IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Serial No.:	10/539,670
Applicant/Appellant:	Gore, T. *et al.*
Confirmation No.:	4798
International File Date:	12/18/03
Group Art Unit:	1648
Examiner:	Sharon L. Hurt
For:	TRIVALENT VACCINE WITH MATERNAL ANTIBODY TRANSFER VIA THE MILK
Attorney Docket:	I-2002.025 US

October 20, 2008

APPEAL BRIEF

Mail Stop: Appeal
Board of Patent Appeals
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Dear Sir/Madam:

Pursuant to Appellant's June 19, 2008 Notice of Appeal, Appellant appeals the claim rejections from the February 19, 2008 final Office action. In support of this appeal, Appellant provides the following information, argument, and fee in accordance with 37 C.F.R. §41.37 and MPEP §§1205 and 1205.02.

Petition for Extension of Time

Appellant respectfully requests a two month extension of time for filing this Appeal Brief. The Commissioner is hereby authorized to charge the fee(s) for such an extension to Deposit Account No. **02-2334**.

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I. REAL PARTY IN INTEREST (37 C.F.R. §41.37(c)(1)(i))

The real party in interest in this appeal is Intervet International B.V. This ownership is evidenced by assignment documents recorded at Reel 018490, Frame 0365 (recorded on November 8, 2006); and at Reel 017181, Frame 0160 (recorded on June 14, 2005).

II. RELATED APPEALS AND INTERFERENCES (37 C.F.R. §41.37(c)(1)(ii))

Appellant is not aware of any prior or pending appeal, judicial proceeding, or interference that may be related to, directly affect, or be directly affected by or have bearing on the Board's decision in this appeal.

III. STATUS OF CLAIMS (37 C.F.R. §41.37(c)(1)(iii))

A total of 42 claims have been introduced in this patent application. As of February 19, 2008, claims 1-27 were cancelled, and claims 28-42 were pending and rejected. However, Appellant filed an After Final Amendment on August 4, 2008, which has not been addressed by the Examiner. Per the August 4, 2008 amendment, claims 1-27, 30, 32, and 38-39 are cancelled and claims 28-29, 31, 33-37 and 40-42 are pending and rejected. Every pending claim has been finally rejected. This appeal requests reversal of all rejections relating to claims 28-29, 31, 33-37 and 40-42, as presented in Appellant's August 4, 2008 amendment.

IV. STATUS OF AMENDMENTS (37 C.F.R. §41.37(c)(1)(iv))

Appellant filed one amendment after the February 19, 2008 final Office action. That amendment, was filed after a July 31, 2008 telephonic interview among Appellant's below signed attorney, Examiner Sharon Hurt and Supervisory Examiner Bruce Campell. During that interview, claim amendments were proposed by and/or discussed with Supervisory Examiner Bruce Campell to bring the claims towards allowance. Indeed, this is reflected in an Interview Summary mailed on August 1, 2008 stating that Appellant "discussed possible claim amendments to be filed after Final to move closer towards allowance." Appellant's After Final Amendment filed on August 4, 2008 reflects the proposals made during the interview. These narrowing claim amendments do not expand the scope of the search required for examination of the claims, and are sought to be entered to place the application into better condition for appeal.

Appellant notes that an advisory action was never issued in response to Appellant's August 4, 2008 After Final Amendment. Hence, it is Appellant's understanding that the claim amendments have not been entered. Nonetheless, Appellant files this appeal based upon the claims as presented in the August 4, 2008 After Final Amendment.

V. SUMMARY OF CLAIMED SUBJECT MATTER (37 C.F.R. §41.37(c)(1)(v))

The embodiments in the pending claims stem from the inventors' discovery of a vaccine comprising a component protective against minute virus of canine (MVC, also termed canine parvovirus type-1, or CPV-1). *See, e.g.*, Appellant's specification, page 9, lines 2-6

There are 2 independent claims (*i.e.*, claims 28 and 34). They are summarized as follows:

Claim 28 is directed to a vaccine comprising an immunogenically effective amount of an inactivated whole Minute virus of canine (MVC, also known as Canine Parvovirus-1 (CPV-1)). Claim 28 is generally supported by Appellant's specification at, for example, page 9, lines 2-6.

Claim 34 is directed to a method of protecting a puppy against Minute virus of canine (MVC, also known as Canine Parvovirus-1 (CPV-1)) comprising

i) administering a vaccine comprising an immunogenically effective amount of an inactivated whole MVC to a pregnant bitch prior to whelp, and

ii) administering colostrums of the bitch to at least one puppy within about forty-eight (48) hours of whelp whereby maternal antibodies are transferred at a sufficiently high titer to protect the puppy from disease caused by MVC.

Claim 34 is generally supported by Appellant's specification at, for example, page 9, lines 12-17, and at page 12, lines 17-25.

VI. GROUNDS OF REJECTION TO BE REVIEWED ON APPEAL (37 C.F.R. §41.37(c)(1)(vi))

There are presently three rejections set forth under 35 U.S.C. §103(a). These are as follows:

A. Claim 28 has been rejected under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli *et al.*, *J. Vet. Diag. Invest. 11*: 365-367 (1999) (hereafter "Pratelli *et al.*").[1]

B. Claims 28, 29, 31 and 33 have been rejected under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli *et al.* and further in view of U.S. 6,159,477.[2]

C. Claims 28, 29, 31, 33-37 and 40-42 (*i.e.*, all of the claims) have been rejected under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli *et al.* and U.S. 6,159,477, and further in view of Poulet *et al., Vet. Rec. 148:*691-695 (2001) (hereafter "Poulet *et al.*") and Correa, J.E., "Canine Breeding and Reproduction," UNP-52, published through the Alabama Cooperative Extension System, Alabama A&M and Auburn Universities, November 2002, 7 pages (hereafter "Correa").[3]

Appellant respectfully appeals all of these rejections.

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[1] Appellant notes that the Examiner's final rejection under 35 U.S.C. §103(a) in light of U.S. 4,193,990, U.S. 4,193,991 and Pratelli *et al.* pertained to claims 28, 30 and 32. *See* page 2 of Detailed Action, mailed February 19, 2008. This appeal, however, is limited to claim 28 as claims 30 and 32 are sought to be cancelled by way of Appellant's After Final Amendment filed on August 4, 2008.

[2] Appellant notes that the Examiner's final rejection under 35 U.S.C. §103(a) in light of U.S. 4,193,990, U.S. 4,193,991, Pratelli *et al.* and U.S. 6,159,477 pertained to claims 28-33. *See* page 3 of Detailed Action, mailed February 19, 2008. This appeal, however, is limited to claims 28, 29, 31 and 33 as claims 30 and 32 are sought to be cancelled by way of Appellant's After Final Amendment filed on August 4, 2008.

[3] Appellant notes that the Examiner's final rejection under 35 U.S.C. §103(a) in light of U.S. 4,193,990, U.S. 4,193,991, Pratelli *et al.*, U.S. 6,159,477, Poulet *et al.* and Correa pertained to claims 28-42. *See* pages 3-4 of Detailed Action, mailed February 19, 2008. This appeal, however, is limited to claims 28, 29, 31, 33-37 and 40-42 as claims 30, 32, 38 and 39 are sought to be cancelled by way of Appellant's After Final Amendment filed on August 4, 2008.

VII. ARGUMENT (37 C.F.R. §41.37(c)(1)(vii))

A. Response to rejection of Claim 28 under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli et al

Claim 28 has been rejected under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli *et al.* Appellant requests reversal of this rejection.

Claim 28 is directed to a vaccine comprising an immunogenically effective amount of an inactivated whole Minute virus of canine (MVC, also known as Canine Parvovirus-1 (CPV-1)). In contrast, the publications applied in the rejection at most only refer to vaccines of Canine Parvovirus-2 (CPV-2), which is an entirely different virus bearing no relationship to MVC/CPV-1.

MVC is a totally different virus than CPV-2. Attached as Appendix B are pages from the third edition (2006) of Craig E. Greene's authoritative treatise "Infectious Diseases of the Dog and Cat." This material was previously included as Exhibit A, appended to Appellant's Response to Non-Final Office Action, filed on November 19, 2007. On page 70, under **Etiology**, it states that "CPV-1 [a.k.a. MVC] is distinctly differentiated from CPV-2 by its host cell range, spectra of hemagglutination, genomic properties, and antigenicity. . . . CPV-1 and CPV-2 are different viruses; no homology in DNA-restriction sites between the two viruses has been demonstrated using several restriction enzymes." Under **Diagnosis**, it states that "CPV-1 [a.k.a. MVC] will not cross react with any of the serologic or fecal detection methods for CPV-2." *Id.*

That MVC/CPV-1 represents an entirely different virus from CPV-2 is corroborated by the documents identified by the Examiner in a Notice of References Cited PTO-892 form accompanying the Office Action mailed on June 29, 2006. Schwartz, D. *et al., Virology 302*: 219-223 (2002) ("Schwartz *et al.*") (provided here as Appendix C) states that "MVC . . .[is] antigenically and genetically distinct form the canine parvovirus type-2 (CPV) based on antibody cross reactivity and restriction enzyme analysis of the viral DNA . . . ." See page 219, left column, first paragraph. Moreover, as evident from Schwartz *et al.*, the skilled artisan perceives MVC/CPV-1 to be distinct from CPV-2: "Here we show that MVC is a distinct member of the Parvoviridae which is most closely related to the bovine parvovirus,

although it shares only 43% identity in DNA sequence with that virus." See page 222, top of left column, emphasis added.

There is a clear genetic difference between MVC/CPV-1 and CPV-2. In fact, Schwartz *et al.* seems to suggest in the last paragraph in the left column of page 222 a better way of grouping the class of parvoviruses: i) adenoassociated viruses, ii) rodent virus-related viruses, and iii) erythroviruses. Whereas the second group includes canine parvovirus (i.e., CPV-2), the authors include MVC in the last group. Moreover, the authors caution that this grouping is imperfect because "MVC is still only distantly related to the other viruses, indicating that it diverged in the distant past." *See* sentence bridging left and right columns on page 222.

Other publications cited by the Examiner also corroborate the fact that MVC is a totally different virus than CPV-2. Pratelli *et al., J. Vet. Diag. Invest. 11*: 365-7 (1999) was cited by the Examiner in the PTO-892 form accompanying the Office Action mailed on March 21, 2007. This publication (provided here as Appendix D) states that "[a]ntigenic and genomic properties of MVC are distinct from those of canine parvovirus type 2 (CPV-2). . . ." *See* page 365, left column. Truyen, U., Recent Advances in Canine Infectious Diseases, International Veterinary Information Service (January 2000) was also cited by the Examiner in the same PTO-892 form. This publication (provided here as Appendix E) states that "[t]wo distinct parvoviruses (CPV), are now known to infect dogs- the pathogenic CPV-2, . . . and the 'minute virus of canines' (MVC, CPV-1). . . . MVC, *a completely different parvovirus*, had not been associated with natural disease until 1992." *See* first paragraph, emphasis added.

That minute virus of canines (MVC) has no relationship to canine parvovirus type 2 (CPV-2) is also exemplified by the fact that they are grouped into separate genera by the International Committee on Taxonomy of Viruses (ICTV). The ICTV's taxonomic structure is available through the U.S. National Institutes of Health's website, and was previously provided by Appellant as Exhibit A in their Statement of the Substance of an Interview and After Final Amendment, filed August 4, 2008. A printout of this taxonomic classification scheme is provided here as Appendix F. This classification identifies MVC to be a species of the bocavirus genus and CPV-2 to be a species of the parvovirus genus.

It is not clear why MVC has been referred to as CPV-1 (canine parvovirus type 1). Perhaps MVC was at one point labeled as a "parvovirus" simply because the Latin prefix "parvo" means small. This notion was previously suggested and corroborated by Exhibit B of Appellant's aforementioned August 4, 2008 filing (provided here as Appendix G). Hence, it appears that MVC and CPV-2 only share the property of being small.

Smallness, however, is not sufficient to demonstrate predictability of success to one of ordinary skill in the art for showing obviousness of the pending claims, as required by the U.S. Supreme Court in their decision KSR International Co. v. Teleflex Inc. 82 U.S.P.Q.2d 1385; *see also* USPTO Examination Guideline "Patent Corps-Wide Training: Determining Obviousness Under 35 U.S.C. §103 After KSR International Co. v. Teleflex Inc." at www.uspto.gov; *see also* 72 Fed. Reg. 57526. That is, the fact that CPV-2 vaccines are in the prior art does not provide the requisite evidence that the skilled artisan would predict that a vaccine could be made for MVC, as CPV-2 and MVC are not related.

The principal basis of the present rejection alleging the obviousness of Appellant's claimed vaccine stems from mistakenly assuming that biological similarities exists between MVC/CPV-1 and CPV-2. Indeed, several publications clearly indicate that skilled artisans recognize that MVC/CPV-1 and CPV-2 are entirely different viruses with very little to nothing in common: they are genetically and antigenically distinct and diverged from each other in the distant past. CPV-2 has been widely studied to the point where numerous vaccines are on the market. In contrast, prior to Appellant's invention, no MVC vaccines were available. Hence, it is unreasonable to expect that MVC can be made into a vaccine simply because vaccines exist for CPV-2. Moreover, the mere fact that a virus is known does not render obvious a vaccine based upon that virus. Indeed, many viruses exist (e.g., human immunodeficiency virus, hepatitis C virus) for which no vaccine has been developed despite intensive research.

Accordingly, a *prima facie* case of obviousness under 35 U.S.C. §103(a) has not been set forth for claim 28, and Appellant respectfully requests reversal of this rejection.

B. Response to rejection of Claims 28, 29, 31 and 33 under 35 U.S.C. §103(a) as being upatentable over U.S. 4,193,990 and U.S. 4,193,991 in view of Pratelli et al. and further in view of U.S. 6,159,477

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not render obvious a vaccine based upon that virus. Indeed, many viruses exist (e.g., human immunodeficiency virus, hepatitis C virus) for which no vaccine has been developed despite intensive research.

Accordingly, a *prima facie* case of obviousness under 35 U.S.C. §103(a) has not been set forth for claims 28, 29, 31, 33-37 and 40-42 and Appellant respectfully requests reversal of this rejection.

VIII. DESCRIPTION OF CLAIMS APPENDIX (37 C.F.R. §41.37(c)(1)(viii))

An appendix containing a copy of all the claims involved in the appeal is attached.

IX. DESCRIPTION OF EVIDENCE APPENDIX (37 C.F.R. §41.37(c)(1)(ix))

Appendices B-G support Appellant's assertion that the skilled artisan regards MVC/CPV-1 as unrelated to CPV-2. The publications provided in these appendices were previously cited by Appellant or the Examiner, as indicated below.

Appendix B contains pages from the third edition (2006) of Craig E. Greene's authoritative treatise "Infectious Diseases of the Dog and Cat." This material was previously included as Exhibit A, appended to Appellant's Response to Non-Final Office Action, filed on November 19, 2007.

Appendix C contains Schwartz, D. *et al., Virology 302*: 219-223 (2002) ("Schwartz *et al.*"). This publication was previously cited by the Examiner in a Notice of References Cited PTO-892 form accompanying the Office Action mailed on June 29, 2006.

Appendix D contains Pratelli *et al., J. Vet. Diag. Invest. 11*: 365-7 (1999). This publication was cited by the Examiner in the PTO-892 form accompanying the Office Action mailed on March 21, 2007.

Appendix E contains Truyen, U., Recent Advances in Canine Infectious Diseases, International Veterinary Information Service (January 2000). This publication was cited by the Examiner in the PTO-892 form accompanying the Office Action mailed on March 21, 2007.

Appendix F contains the International Committee on Taxonomy of Viruses' (ICTV). taxonomic structure of the Parvoviridae family, available from the U.S. National Institutes of Health's website at www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm, and was previously provided by Appellant as Exhibit A in their Statement of the Substance of an Interview and After Final Amendment, filed August 4, 2008.

Appendix G contains a definition for the Latin prefix "parvo," available from www.wordinfo.info. A printout of this definition was previously provided as Exhibit B of Appellant's August 4, 2008 filing.

X. DESCRIPTION OF RELATED PROCEEDINGS APPENDIX H (37 C.F.R. §41.37(c)(1)(x))

None.

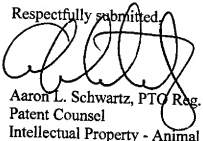
XI. Fee payment and extension request

Appellant authorizes the Commissioner to charge Deposit Account No. **02-2334** for the fee under 37 CFR §41.20(b)(2) for filing this appeal. Appellant does not believe that any other fee is due in connection with this filing. If, however, Appellant does owe any such fee(s), the Commissioner is hereby authorized to charge the fee(s) to Deposit Account No. **02-2334**. In addition, if there is ever any other fee deficiency or overpayment in connection with this patent application, the Commissioner is hereby authorized to charge such deficiency or overpayment to Deposit Account No. **02-2334**.

* * * * * * * * *

Appellant submits that the pending claims are in condition for allowance, and requests the rejections in February 19, 2008 final Office action be reversed, and this application be allowed.

Respectfully submitted,



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APPENDIX A
Claims Appendix (37 C.F.R. §41.37(c)(1)(viii))

1.-27. (cancelled)

28. A vaccine comprising an immunogenically effective amount of an inactivated whole Minute virus of canine (MVC, also known as Canine Parvovirus-1 (CPV-1)).

29. The vaccine of claim 28 further comprising at least one additional antigen selected from the group consisting of a canine herpesvirus (CHV) antigen, a canine rotavirus (CRV) antigen, and a Canine Parvovirus type 2 (CPV-2) antigen.

30. (cancelled)

31. The vaccine of claim 29 wherein the at least one additional antigen is an inactivated virus.

32. (cancelled)

33. The vaccine of claim 29 wherein the at least one additional antigen is an attenuated live virus.

34. A method of protecting a puppy against Minute virus of canine (MVC, also known as Canine Parvovirus-1 (CPV-1)) comprising

i) administering a vaccine comprising an immunogenically effective amount of an inactivated whole MVC to a pregnant bitch prior to whelp, and

ii) administering colostrums of the bitch to at least one puppy within about forty-eight (48) hours of whelp whereby maternal antibodies are transferred at a sufficiently high titer to protect the puppy from disease caused by MVC.

35. The method of claim 34, comprising administering colostrums of the bitch to at least one puppy within about 24 hours of whelp.

36. The method of claim 34, wherein the maternal antibodies are transferred by allowing the puppy to nurse the bitch within about forty-eight (48) hours of whelp.

37. The method of claim 36, wherein the maternal antibodies are transferred by allowing the puppy to nurse the bitch within about 24 hours of whelp.

38-39. (cancelled)

40. The method of claim 34, wherein the vaccine further comprises at least one additional antigen selected from the group consisting of a canine herpesvirus (CHV) antigen, a canine rotavirus (CRV) antigen, and a Canine Parvovirus type 2 (CPV-2) antigen.

41. The method of claim 40, wherein the at least one additional antigen is an inactivated virus.

42. The method of claim 40, wherein the at least one additional antigen is an attenuated live virus.

APPENDIX B

Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix B contains pages from the third edition (2006) of Craig E. Greene's authoritative treatise "Infectious Diseases of the Dog and Cat." This material was previously included as Exhibit A, appended to Appellant's Response to Non-Final Office Action, filed on November 19, 2007.

Infectious Diseases

OF THE DOG AND CAT

THIRD EDITION

Craig E. Greene, DVM, MS, DACVIM

Professor Emeritus, and Josiah Meigs Distinguished Teaching Professor
Department of Infectious Diseases and Department of Small Animal Medicine
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6. Evermann J. 1985. Paramyxovirus infections of dogs. *Vet Rec* 117:450-451.
9. Macartney L, Cornwell HJ, McCandlish IA, et al. 1985. Isolation of a novel paramyxovirus from a dog with enteric disease, *Vet Rec* 117:205-207.
12. Vieler E, Herbst W, Baumgartner W, et al. 1994. Isolation of a parainfluenza virus type 2 from the prostatic fluid of a dog, *Vet Rec* 135:384-385.

*See the CD-ROM for a complete list of references.

CHAPTER • 8

Canine Viral Enteritis

Dudley L. McCaw and Johnny D. Hoskins

Since the late 1970s, viral enteritis has become recognized as one of the most common causes of infectious diarrhea in dogs younger than 6 months. Canine parvovirus (CPV)–1 and –2), canine coronavirus (CCV), and canine rotaviruses (CRVs) have been incriminated as primary pathogens. Astrovirus, herpesvirus, enteroviruses, calicivirus, parainfluenza viruses, and virus-like particles have been isolated from or identified in feces from dogs with diarrhea, but their pathogenicity is uncertain.[21,29,31]

CANINE PARVOVIRAL ENTERITIS

Etiology

CPVs are small, nonenveloped, DNA-containing viruses that require rapidly dividing cells for replication (Fig. 8-1). As is the case with all parvoviruses, CPV-2 and -1 are extremely stable and are resistant to adverse environmental influences. CPV-2 is known to persist on inanimate objects, such as clothing, food pans, and cage floors, for 5 months or longer.

Most common detergents and disinfectants fail to inactivate CPVs. A noteworthy exception is sodium hypochlorite (1 part common household bleach to 30 parts water), which is an effective and inexpensive disinfectant. It is important that exposure to this disinfectant be prolonged (at least 10 minutes) and thorough.

Canine parvoviral enteritis is probably one of the most common infectious disorders of dogs. This highly contagious, often fatal disease is caused by CPV-2. Since its emergence in the late 1970s, CPV-2 has undergone genetic alterations in the dog, with development of new strains of the virus.[20,81,89] In 1980, the original strain of CPV-2 evolved into type 2a (CPV-2a); and in 1984, another variant designated type 2b (CPV-2b) appeared. These CPV-2 alterations were associated with a genetic adaptation, enabling the parvovirus to replicate and spread more effectively in susceptible dogs. In the United States and Japan, CPV-2b has largely replaced those previously isolated strains, whereas in the Far East[80,60] and Europe,[17,26] both CPV-2a and -2b predominate.[55] In 2000, another strain was reported (CPV-2c), which was an adaptation that allowed infection of cats.[41] Although CPV-2c has been isolated only from leopard cats, infection in domestic cats and dogs is likely.[41] Genetic mutations in the structure of the surface transferrin receptor (TfR) of the virus has resulted in structural alterations that control the host adaptation of CPV strains.[41] For a further discussion of CPV strains in cats, see Canine Parvovirus Infection of Cats in Chapter 10.

Epidemiology

Natural CPV-2 infections have been reported in domestic dogs, bush dogs (*Speothos venaticus*), coyotes (*Canis latrans*), crab-eating foxes (*Cerdocyon thous*), and maned wolves (*Chrysocyon brachyurus*); and most if not all Canidae are susceptible. Experimental infections can be produced in domestic ferrets, mink, and cats; however, the infection is generally self-limiting. The original CPV-2 isolates produced only systemic and intestinal infections in dogs,[128] whereas the newer type 2a and 2b strains may infect felines under experimental[68,72,131] and natural[71,127] circumstances (see Chapter 10). In domestic dogs, CPV-2 infection does not necessarily result in apparent disease; many dogs that become naturally infected never develop overt clinical signs. When the disease occurs, clinical illness is most severe in young, rapidly growing pups that harbor intestinal helminths, protozoa, and certain enteric bacteria such as, *Clostridium perfringens, Campylobacter* spp., and *Salmonella* spp. In susceptible animals, the incidence of severe disease and death can be very high.

CPV-2 is highly contagious, and most infections occur as a result of contact with contaminated feces in the environment. In addition, people, instruments (equipment in veterinary facilities or grooming operations), insects, and rodents can serve as vectors. Dogs may carry the virus on their hair coat for extended periods. The incubation period of CPV-2 in the field is 7 to 14 days; experimentally, the incubation period has been found to be 4 to 5 days. With CPV-2a and -2b strains, the incubation period in the field can be as brief as 4 to 6 days.

Acute CPV-2 enteritis can be seen in dogs of any breed, age, or sex. Nevertheless, pups between 6 weeks and 6 months of age, and Rottweilers, Doberman pinschers, Labrador retrievers, American Staffordshire terriers, German shepherds, and Alaskan sled dogs seem to have an increased risk.[85,86]

Pathogenesis

CPV-2 spreads rapidly from dog to dog via oronasal exposure to contaminated feces (Fig. 8-2). Virus replication begins in lymphoid tissue of the oropharynx, mesenteric lymph nodes, and thymus and is disseminated to the intestinal crypts of the

small intestine by means of viremia. Marked plasma viremia is observed 1 to 5 days after infection. Subsequent to the viremia, CPV-2 localizes predominantly in the gastrointestinal (GI) epithelium lining the tongue, oral and esophageal mucosae, and small intestine and lymphoid tissue, such as thymus, lymph nodes, and bone marrow. It may also be isolated from the lungs, spleen, liver, kidney, and myocardium.[128]

Normally, intestinal crypt epithelial cells mature in the small intestine and then migrate from the germinal epithelium of the intestinal crypts to the tips of the villi (Fig. 8-3, *A*). After reaching the villous tips, the intestinal epithelial cells acquire their absorptive capability and aid in assimilating nutrients. Parvovirus infects the germinal epithelium of the intestinal crypts, causing destruction and collapse of the epithelium (see Fig. 8-3, *B*). As a result, normal cell turnover (usually between 1 and 3 days in the small intestine) is impaired, and the villi become shortened. CPV-2 also destroys mitotically active precursors of circulating leukocytes and lymphoid cells. In severe infections, the results are often neutropenia and lymphopenia. Secondary bacterial infections from gram-negative and anaerobic microflora cause additional complications related to intestinal damage, bacteremia and endotoxemia, and disseminated intravascular coagulation (DIC).[84,128,136] Active excretion of CPV-2 begins on the third or fourth day after exposure, generally before overt clinical signs appear. CPV-2 is shed extensively in the feces for a maximum of 7 to 10 days. Development of local intestinal antibody is most likely important in the termination of fecal excretion of parvovirus. Serum antibody titers can be detected as early as 3 to 4 days after infection and may remain fairly constant for at least 1 year.

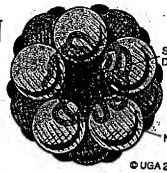

Fig 8-1 Structure of parvovirus. (Courtesy University of Georgia, Athens, Ga.)

Clinical Findings

CPV-2 infection has been associated with two main tissues—GI tract and myocardium—but the skin and nervous tissue can also be affected. In addition, other clinical complications of secondary infection or thrombosis can occur. A marked vari-

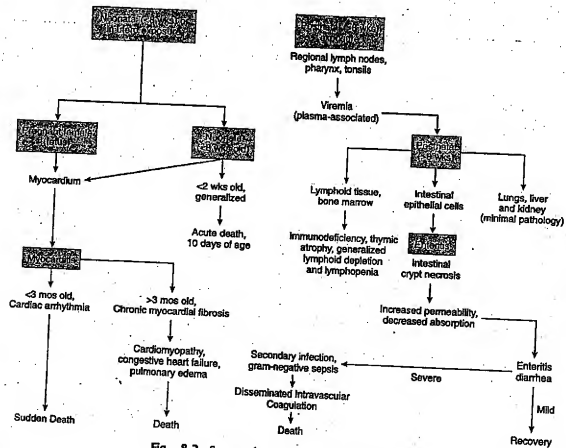

Fig 8-2 Sequential pathogenesis of CPV-2 infection.

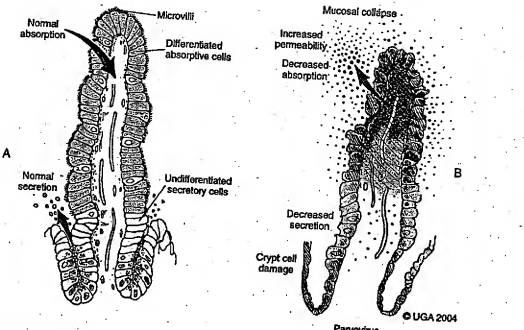

Fig 8-3 A, Normal intestinal villus showing cellular differentiation along the villus. B, Parvovirus-infected villus showing collapse and necrosis of intestinal villus. (Courtesy University of Georgia, Athens, Ga.)

ation is found in the clinical response of dogs to intestinal infection with CPV-2, ranging from inapparent infection to acute fatal disease. Inapparent, or subclinical, infection occurs in most dogs. Severity of the CPV-2 enteritis depends on the animal's age, stress level, breed, and immune status. The most severe infections are usually in pups younger than 12 weeks because these pups lack protective immunity and have an increased number of growing, dividing cells.

Parvoviral Enteritis

CPV-2 enteritis may progress rapidly, especially with the newer strains of CPV-2. Vomiting is often severe and is followed by diarrhea, anorexia, and rapid onset of dehydration. The feces appear yellow-gray and are streaked or darkened by blood (Fig. 8-4). Elevated rectal temperature (40° to 41° C [104° to 105° F]) and leukopenia may be present, especially in severe cases. Death can occur as early as 2 days after the onset of illness and is often associated with gram-negative sepsis or DIC, or both. Younger age, neutropenia, and Rottweiler breed have been associated with a poorer chance of survival (unpublished data).[31]

Fig 8-4 Dog with severe bloody diarrhea characteristic of severe parvoviral enteritis. (Courtesy University of Georgia, Athens, Ga.)

Neurologic Disease

Primary neurologic disease may be caused by CPV-2 but more commonly occurs as a result of hemorrhage into the central nervous system (CNS) from DIC or from hypoglycemia during the disease process, sepsis, or acid-base-electrolyte disturbances.[1] Concurrent infection with viruses such as canine distemper virus is also possible. Cerebellar hypoplasia, common in kittens prenatally or neonatally infected with feline panleukopenia virus, has not been adequately reported in pups with CPV-2 infection. CPV DNA was amplified using polymerase chain reaction (PCR) from brain tissue of two dogs with cerebellar hypoplasia, but time of exposure to CPV was not mentioned.[114] CPV-2 has been identified in the CNS of cats (see Central Nervous System Infection in Chapter 10).

Cutaneous Disease

Erythema multiforme was diagnosed in a dog with parvoviral enteritis.[20] Skin lesions included ulceration of the footpads, pressure points, and mouth and vaginal mucosa. Vesicles in the oral cavity and erythematous patches on the abdomen and perivulvar skin were also present. Parvovirus was confirmed in the affected cells by immunohistochemistry.

Canine parvovirus–2 Myocarditis

CPV-2 myocarditis can develop from infection in utero or in pups younger than 8 weeks. All pups in a litter are usually affected. Pups with CPV-2 myocarditis often die, or they succumb after a short episode of dyspnea, crying, and retching. Signs of cardiac dysfunction may be preceded by the enteric form of the disease or may occur suddenly, without apparent previous illness. The spectrum of myocardial disease in individuals is wide and may include any of the following: acute diarrhea and death, without cardiac signs; diarrhea and apparent recovery followed by death, which occurs weeks or months later as a result of congestive heart failure; or sudden onset of congestive heart failure, which occurs in apparently normal pups at 6 weeks to 6 months of age. Myocarditis is still occasionally found in pups born to isolated, unvaccinated bitches[135] in contrast to its frequent occurrence during the widespread epizootic outbreaks of the late 1970s in CPV-naive dogs.[1] Myocarditis, with or without enteritis, has been associated with natural CPV-2a and -2b infections in 6- to 14-week-old dogs from Korea.[139] CPV infection appears not to be a common cause of heart disease because PCR analysis at necropsy of 271 dogs with either dilated cardiomyopathy or myocarditis did not detect CPV in any of the samples.[63]

Thrombosis

Dogs with naturally occurring CPV-2 infections have clinical and laboratory evidence of hypercoagulability.[86] These dogs may develop thrombosis or phlebitis with catheters or visceral thrombi.

Bacteriuria

Asymptomatic urinary tract infection has been detected in approximately 25% of pups following CPV-2 enteritis.[30] This predisposition was attributed to fecal contamination of the external genitalia in association with neutropenia. Untreated subclinical urinary tract infection may lead to chronic urinary infection as an undesirable consequence.

Intravenous Catheter Infection

Bacteria from GI or environmental origin have been isolated from the intravenous (IV) catheters removed from dogs being treated for suspected parvoviral infections.[55] Most of these organisms were gram-negative types (*Serratia, Acinobacter, Citrobacter, Klebsiella,* and *Escherichia*). Most organisms were resistant to penicillins, first-generation cephalosporins, and macrolides while being susceptible to aminoglycosides, fluoroquinolones, chloramphenicol, potentiated sulfonamides, and clavulanate-potentiated penicillins. Despite the positive culture results of the catheter tips, none of the dogs showed systemic clinical signs of infection, and only one developed local phlebitis.

Diagnosis

The sudden onset of foul-smelling, bloody diarrhea in a young (under 2 years) dog is often considered indicative of CPV-2 infection. However, all dogs with bloody diarrhea (with or without vomiting) are not infected necessarily with CPV-2. Other enteropathogenic bacterial infections should also be considered (see Chapter 39). All clinical signs characteristic of CPV-2 infection are seldom present at any one time. Leukopenia, although not found in all dogs, is usually proportional to the severity of illness and the stage of disease at the time the blood is taken. Abnormal coagulation test results may include prolongation of the activated partial thromboplastin time, increased thromboelastogram amplitude, and decreased antithrombin III activity.[86]

Fecal enzyme-linked immunosorbent assay (ELISA) antigen tests are available for in-hospital testing for CPV-2 infection (see Appendix 6). These tests are relatively sensitive and specific for detecting CPV-2 infection.[38,44,45,52] However, the period of fecal virus shedding is brief; CPV-2 is seldom detectable by 10 to 12 days after natural infection. This corresponds to 5 to 7 days of clinical illness. Positive results confirm infection or may be induced by all attenuated live CPV-2 vaccines (vaccine virus can yield a false-positive result in dogs 5 to 12 days after vaccination); negative results do not eliminate the possibility of CPV-2 infection. Generally, vaccine-induced reactions are weak positive compared with natural infection.

CPV-2 typically produces lesions in the jejunum, ileum, mesenteric lymph nodes, and other lymphoid tissues. CPV-2 can be isolated from these tissues or feces using tissue culture systems, if performed early. Later in the course of disease, virions become coated by antibodies and cleared. In most tissues, intranuclear inclusions are observed. In the glossal epithelium, these may appear as being within the cytoplasm, when in fact they originate in the nuclear space.[42] Immunochemical methods can also be used to detect virus in tissue culture, electron microscopy (EM) scan of feces or tissues (see Pathologic Findings in this chapter). PCR has been used as a specific and sensitive means of detecting CPV in feces of infected dogs.[68,72,132] This method can also help to differentiate between virulent and vaccine CPV strains.[118]

As a general rule, parvoviruses cause hemagglutination of erythrocytes. Inhibition of hemagglutination by CPV-2 antisera can be used to demonstrate serum antibody. The presence of high hemagglutination inhibition (HI) titer in a single serum sample collected after the dog has been clinically ill for 3 or more days is diagnostic for CPV-2 infection. Rising titers (seroconversion) can also be demonstrated when acute and 10- to 14-day convalescent serum samples are compared using either canine or feline parvovirus in HI and virus neutralization (VN) tests. ELISA tests are also available that permit distinction between IgG and IgM.[111] In-office ELISA test kits are commercially available for semiquantitative IgG and IgM measurements (Immunocomb, Biogal Labs, Megiddo, Israel)[137,138] and for determining adequate IgG titers for vaccination (CPV/CAV SNAP Test Kit, Synbiotics, San Diego, Calif.). See Appendix 6 for further information on these products.

Pathologic Findings

Early lesions are most pronounced in the distal duodenum; later, the jejunum is more severely affected. The intestinal wall is generally thickened and segmentally discolored, with denudation of intestinal mucosa and the presence of dark, sometimes bloody, watery material within the stomach and intestinal lumen (Fig. 8-5). In mild cases, the lesions are not easy to distinguish from those of nonspecific enteritis. Enlargement and edema of thoracic or abdominal lymph nodes have been observed.

The intestinal lesions are characterized by necrosis of the crypt epithelium in the small intestine. Intranuclear viral inclusion bodies may be seen in these epithelial cells and throughout the squamous epithelia of the upper GI tract.[62] The pathologic changes may range from mild inflammation to diffuse hemorrhagic enteritis. The villi are shortened or obliterated, owing to lack of epithelial replacement by maturing crypt cells, resulting in collapse of the lamina propria (Fig. 8-6). Necrosis and depletion of the lymphoid tissue (e.g., Peyer's patches; mesenteric lymph nodes, thymus, and spleen) are present. Pulmonary edema or alveolitis may be observed in dogs dying of complicating septicemia.[130] Histologic examination is usually definitive; however, specific identification of parvovirus in tissue specimens can be done by immunofluorescence or other immunochemical methods. Using indirect fluorescent antibody (FA) testing, antigen in dogs with

Fig 8-5 Small intestine at necropsy from a dog that died suddenly of parvoviral enteritis. Note the discoloration of the intestinal wall and fibrin on the serosal surfaces. (Courtesy Veterinary Pathology, University of Georgia, Athens, Ga.)

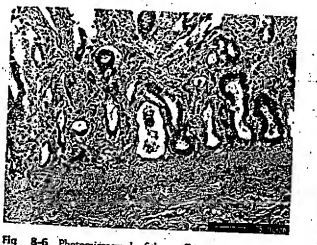

Fig 8-6 Photomicrograph of the small intestine of a dog that died of parvoviral enteritis. Villi are collapsed, and crypt lumina are dilated and filled with necrotic debris (H and E stain, ×100). (Courtesy Barry Harmon, University of Georgia, Athens, Ga.)

lethal CPV enteritis can be found in the dorsal side of the tongue (96.3%), pharynx (81%), esophagus (50%), ventral tongue (20.4%), planum nasale (5.6%), small intestinal mucosa (85.2%), bone marrow (81.6%), spleen (79.6%), thymus (66.7%), mesenteric nodes (50.4%), palatine tonsils (58.5%), and myocardium (1.9%).[138] In situ hybridization is a valuable specific tool for virus identification in formalin-fixed or wax-embedded tissue specimen.[139]

Parvoviral myocarditis, when present, is recognized grossly as pale streaks in the myocardium (Fig. 8-7). The myocardial lesions consist of a nonsuppurative myocarditis with multifocal infiltration of lymphocytes and plasma cells within the myocardium. Basophilic intranuclear inclusion bodies have been observed in cardiac muscle fibers, and parvolike

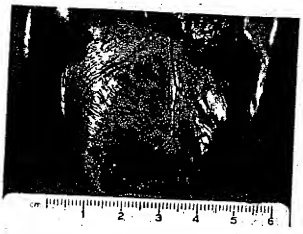

Fig 8-7 Heart from a dog that died of the myocardial form of CPV-2 infection. Pale streaking of the myocardium is apparent. A similar lesion will be noted with CPV-1 infection in puppies younger than 3 weeks. (Courtesy Pfizer Animal Health, Lincoln, Neb.)

virus particles have been demonstrated by EM and by in situ hybridization[135] in the inclusion bodies.

Therapy

The primary goals of symptomatic treatment for CPV-2 enteritis are restoration of fluid and electrolyte balance and preventing secondary bacterial infections. Antimicrobial agents, motility modifiers, and antiemetic agents are given in Table 8-1. Fluid therapy is probably the single most important aspect of clinical management and should be continued for as long as vomiting or diarrhea (or both) persists. Hypoglycemia and hypokalemia are common and should be corrected through additions to the IV fluids. Antimicrobial agents are recommended because the combination of severe disruption of the intestinal epithelium allowing bacteria into the blood and peripheral neutropenia increases the risk of sepsis.[108] The most common bacteria appear to be *Escherichia coli* and *Clostridium perfringens*.[108,109] The best antibacterial spectrum is provided by combination of a penicillin and an aminoglycoside. Before a nephrotoxic drug such as an aminoglycoside is administered, the patient should be fully hydrated. Antiemetic drugs are helpful to reduce fluid loss and decrease patient distress and allows for enteral nutrition. Metoclopramide hydrochloride and prochlorperazine have proved helpful in most dogs with persistent vomiting. The serotonin receptor antagonists are the most efficacious antiemetics.[57,85] Ondansetron and dolasetron have both been used in dogs. Drug therapy to alter gut motility is seldom recommended in the treatment of CPV-2 enteritis. If needed, narcotic antispasmodics (e.g., diphenoxylate hydrochloride, loperamide hydrochloride) are preferred when motility modifiers are needed.

Although withholding food and water are general recommendations in treating GI diseases, including parvovirus enteritis, recent information suggests this is not necessary. When dogs with parvovirus enteritis were fed beginning on the first day of treatment (via nasoesophageal tube), their recovery time was shortened, and they maintained body weight when compared with dogs that were treated the conventional method of withholding food until signs had ceased for 12 hours.[75]

After GI signs abate, a broad-spectrum dewormer and treatment for *Giardia* infection should be given. During the

Table 6-1

Drug Therapy for Canine Viral Enteritis

DRUG	DOSAGE[a] (mg/kg)	ROUTE	INTERVAL (HR)	DURATION (DAYS)
Antiemetic Agents				
Chlorpromazine	0.5	IM	8	prn
	1.0	Rectally	8	prn
	0.05	IV	8	prn
Metoclopramide	0.2–0.4	SC	8	prn
	1–2	IV[b]	24	prn
Prochlorperazine	0.1	IM	6–8	prn
Ondansetron	0.1–0.15	IV	6–12	prn
Dolasetron	1	IV, PO	24	prn
Antimicrobial Agents				
Ampicillin	10–20	IV, IM, SC	6–8	3–5
Cefazolin	22	IV, IM	8	3–5
Ceftiofur	2.2–4.4	SC	12	3–5
Gentamicin	2[c]	IM, SC	8	3–5
Interferon-α	2.5×10^6 units/kg	IV	24	3
Gastric Protectants				
Cimetidine	5–10	IM, IV	6–8	prn
Ranitidine	2–4	SC, IV	6–8	prn
Miscellaneous Therapy				
Whole blood	10–20 ml/kg	IV		prn
Plasma	10–20 ml/kg	IV[d]		prn
Dexamethasone sodium phosphate[e]	2–4	IV		Do not repeat
Flunixin meglumine	1	IV		Do not repeat
Antiendotoxin serum[f]	8.8 ml/kg (diluted in equal amount crystalloid fluid)	IV		Do not repeat
Colloid fluid[g]	20 ml/kg	IV	24	prn

IM, Intramuscular; *IV*, intravenous; *SC*, subcutaneous; *PO*, by mouth, *prn*, as needed.
[a]Dose per administration at specified interval. For additional information on these drugs, see Drug Formulary, Appendix 8.
[b]Slow infusion can be used for severe vomiting.
[c]Administered after correction of dehydration.
[d]Administered over 4 hours.
[e]SEPTI-SERUM; Immvese Inc., Columbia, MO. (Based on a concentration of >320 mg of IgG/mL)
[f]Hetastarch or Dextran 70.

initial stage of CPV-2 enteritis, recommended adjunctive therapy has included transfusion of specific hyperimmune plasma or administration of antiendotoxin sera[18] (see Passive Immunization, Chapter 100, and Drug Formulary, Appendix 8). These adjuncts reportedly decrease mortality and the length of hospitalization[18] but are expensive. A recombinant bactericidal-permeability–increasing (BPI) protein, which counteracts endotoxin, did not alter clinical outcome or survival in dogs naturally infected with CPV-2.[85] This result is despite increases in plasma endotoxin in affected animals.

Recombinant human granulocyte colony-stimulating factor (G-CSF) has been advocated for the treatment of severe neutropenias induced by CPV-2 infection.[22] However, supplementing recombinant human G-CSF to neutropenic pups with CPV-2 infection did not change any aspect of their clinical outcome.[67,109,110] The lack of efficacy of exogenous G-CSF is probably the result of already existent high levels of endogenous G-CSF that are maximally stimulating the production of neutrophils.[11]

Dogs with experimental and natural parvovirus infection have been treated with recombinant feline IFN-ω in high IV dosages (2.5×10^6 units/kg) beginning early (4 days or less after infection) in the course of parvoviral infection.[15, 45,60,66] Reduced signs of clinical illness and mortality were observed in treated dogs. See Drug Formulary, Appendix 8 for further information on its availability and usage.

Several therapies have been recommended and empirically would seem of benefit, but they have not been examined well enough to indicate that they are efficacious.[56] Some puppies are severely anemic, which may be the result of GI loss of blood caused by the parvovirus enteritis, or it might be unrelated to parvovirus such as parasitism. Transfusion of whole blood might benefit these puppies. Hypoproteinemia is present in some puppies. A whole blood transfusion will help resolve the problem, but if erythrocytes are not needed, a more appropriate therapy is plasma transfusion. Ideally, serum albumin concentration should be maintained at 2.0 g/dl or greater. If edema is present as a result of decreased proteins and is not corrected by a plasma transfusion, then synthetic colloid such as hetastarch should be considered. Colloids should not be given until dehydration is corrected. Glucocorticoids and flunixin meglumine may have beneficial effects in

treating early sepsis or endotoxemia. These agents should not be used until dehydration is corrected, and repeated doses should not be given.

The use of hyperimmune plasma might be questioned because, at the time of clinical signs, the levels of antibodies are generally increased. However, pups that had a delayed or lower response are often more severely affected. Canine lyophilized IgG has been beneficial in treatment of dogs with naturally occurring CPV-2 infection.[58] Compared with control dogs, those receiving IgG as adjunctive therapy had reduced severity of disease, reduced cost of treatment, and reduced hospitalization time.

Pups that survive the first 3 to 4 days of CPV-2 enteritis usually make a rapid recovery, generally within 1 week in uncomplicated cases. Severely ill pups that develop secondary sepsis or other complications may require prolonged hospitalization.

Prevention

Immunity After Infection

A puppy that recovers from CPV-2 enteritis is immune to reinfection for at least 20 months and possibly for life. On reexposure to the various strains of CPV-2, protected pups will not have increased serologic titers, show overt signs of illness, or shed virus in the feces. In general, a good correlation exists between serum antibody titer, determined by either HI or VN testing, and resistance to infection. Serum antibody titers remain high for a prolonged period after CPV-2 enteritis, even if reexposure does not occur. If serum antibody titers become low, a localized infection is possible, but viremia and generalized illness are unlikely to develop. Although it may help in protection against entry of CPV-2, intestinal secretory IgA probably does not play a role in the longevity of protective immunity because intestinally derived antibody titers do not persist for longer than 15 days after infection.

Immunization and Duration of Immunity

Inactivated CPV-2 vaccines of sufficient antigenic mass protect dogs against wild-type CPV-2 exposure. If protective immunity is defined as complete resistance to subclinical infection, then that produced by most inactivated CPV-2 vaccines is short lived. Dogs vaccinated with inactivated CPV-2 vaccine can become subclinically infected as early as 2 weeks after vaccination. If a dog is given sequential doses of inactivated CPV-2 vaccine, however, a rapid secondary immune response is mounted, and the dog is protected for as long as 15 months.

Commercially prepared attenuated live and inactivated CPV-2 vaccines are available. These vaccines produce varying levels of protective immunity and are safe either alone or in combination with other vaccine components. Transient lymphopenia occurs 4 to 6 days after the administration of some attenuated live CPV-2 vaccines. Most attenuated live CPV vaccine strains replicate in the intestinal tract and are briefly shed in the feces. Although concern has been expressed about the possibility of attenuated CPV-2 vaccine undergoing reversion of virulence and causing apparent disease, experimental studies have shown that modified live virus (MLV) CPV-2 vaccines are safe.[59] The events following administration of attenuated live CPV-2 vaccines parallel those following wild-type CPV-2 infection. On day 2 after subcutaneous (SC) administration of vaccine, viremia and systemic distribution occur with shedding from GI tract on days 3 to 10. One difference between vaccine-induced and wild-type infections is that lower quantities of virus are shed after vaccination. Humoral immune responses to attenuated live vaccines that have been studied are similar to those observed with wild-type infection.

Serum antibody is usually detectable 3 days after vaccination, with levels rising rapidly to those observed after subsequent natural infection. Even if reexposure does not occur, protective antibody titers may persist for at least 2 years, and dogs exposed during this time should not become infected. Vaccination with potentiated attenuated CPV-2 vaccine has been shown to protect dogs on subsequent experimental challenge exposure.[115] On the basis of serum antibody titers, in a veterinary hospital setting, 27% of the dogs being evaluated for revaccination had titers below the protective level for CPV-2.[45] Although serum antibody titers are not absolute indicators of protection, they have a good correlation with protection against CPV-2 infection (see also Canine Parvoviral Infection, Chapter 100). Even systemic chemotherapy for neoplasia in dogs did not affect serum CPV-2 antibody titers.[33]

Attenuated Live CPV-2 Immunization

Contrary to publicized information, vaccination failure is not related to strain differences between field and vaccine strains. The primary causes of failure of vaccines are interfering levels of maternal antibody to CPV-2[82,98] and lack of sufficient seroconversion to the CPV-2 vaccine administered. The age at which pups can be successfully immunized is proportional to the antibody titer of the bitch, effectiveness of colostral transfer of maternal antibody within the first 24 hours of life, and immunogenicity and antigen titer of the CPV-2 vaccine. Pups from a bitch with low protective titer of antibody to CPV-2 can be successfully immunized by 6 weeks of age, but in pups from a bitch with a very high titer to CPV-2, maternal antibody may persist longer.[60]

Without knowledge of the antibody status of each puppy, recommending a practical vaccination schedule that will protect all of them is difficult. In addition, pups become susceptible to wild-type CPV-2 infection 2 to 3 weeks before they can be immunized. No vaccines are available that completely eliminate this window of susceptibility before pups become immunized.[26] With the *potentiated* vaccines presently available, which are more immunogenic than the original or *conventional* CPV vaccines, low levels of maternal antibody will not prevent successful response. Pups of unknown immune status can be vaccinated with a high-titer–attenuated live CPV-2 vaccine at 6, 9, and 12 weeks of age and then revaccinated annually.[37] A check for serum antibody level or an additional vaccination might be done at 15 to 16 weeks of age, especially in breeds that are at increased risk for CPV-2 enteritis.[12] See discussion of parvoviral infection in Chapter 100 for additional information.

Attenuated Live Canine Parvovirus–2b Immunization

Although not currently commercially available, experimental use of a modified live vaccine derived from CPV-2b produced higher antibody titers to CPV-2b and CPV-2 than did a vaccine derived from CPV-2.[102] In addition, the CPV-2b vaccine was able to produce a titer increase in puppies with higher maternal antibody levels.[101]

Experimental Vaccines

A large number of genetically engineered vaccines have been developed in an attempt to improve the protection afforded by inactivated products while reducing the antigenicity of the potentiated vaccines. A DNA vaccine containing a plasmid encoding the full length of the viral protein (VP)1 region of CPV-2 protected 9-month-old pups from clinical signs and fecal shedding of virus experimental challenge-infection.[48] A vaccine based on a recombinant plant virus expressing the VP2 peptide, coded by a subset of the VP1 gene, protected against clinical disease, with limited fecal shedding following challenge.[53] Neither of these vaccines produced sterile immu-

nity as follows attenuated CPV-2 vaccination. Intranasal or SC vaccination of mice with a plant virus expressing a CPV-2 peptide elicited systemic and mucosal antibody responses.[29,30]

Husbandry

CPV is one of the most resistant viruses to infect dogs. As a result, the hair coat and environment of the ill dog become contaminated. Diluted household bleach (1:30) with water should be applied to tolerable surfaces or used as a dip for animals leaving isolation facilities. Bleach should be added to washing of all utensils and bedding. The solutions require a 10-minute minimum exposure time. The shedding period is so short (under 4 to 5 days following the onset of illness) that the environment is of major concern. The virus can persist for months to years away from sunlight and disinfectants. Steam cleaning can be used for instantaneous disinfection of surfaces that do not tolerate hypochlorite. For further information on disinfection, see Chapter 94.

Public Health Considerations

Studies have failed to find any evidence of human infection by CPV-2, even among kennel workers in heavily contaminated premises, although people apparently can act as passive transport vehicles for the virus between dogs. Although CPV-2 is not itself a human pathogen, extra care should always be practiced in handling fecal materials from diarrheic animals.

CANINE PARVOVIRUS-1 INFECTION

Etiology

In 1967, CPV-1 (also referred to as minute virus of canines [MVC]) was first isolated from the feces of military dogs. Physical and chemical properties of CPV-1 are typical of parvoviruses. CPV-1 is distinctly differentiated from CPV-2 by its host cell range, spectra of hemagglutination, genomic properties, and antigenicity.[7] Using genetic analysis, it is most closely related to bovine parvovirus.[117]

CPV-1 can be propagated on the Walter Reed canine (WRC) cell line. By HI tests, CPV-1 is serologically distinct from parvoviruses of a number of other species. Apparently, CPV-1 and CPV-2 are different viruses; no homology in DNA restriction sites between the two viruses has been demonstrated using several restriction enzymes.

Epidemiology

The domestic dog is the only proven host, although other Canidae are likely susceptible. Before 1985, CPV-1 was considered a nonpathogenic parvovirus of dogs. Since that time, clinical infections of CPV-1 in neonatal pups have been encountered by practicing veterinarians and diagnostic laboratory personnel. Serologic evidence indicates that its distribution is widespread in the dog population but is usually restricted to causing clinical disease in pups younger than 3 weeks,[8] but disease has been reported in pups 5 weeks of age.[100] A reasonable assumption is that the spread is similar to that of CPV-2. Although it was first identified in the United States, isolations have been made worldwide,[46] and similar to CPV-2, it is likely ubiquitous.

Pathogenesis

The virulence of CPV-1 for dogs is uncertain; however, it has been identified by immunoelectron microscopy in the feces of pups and dogs with mild diarrhea. Between 4 and 6 days after oral exposure, CPV-1 can be recovered from the small intestine, spleen, mesenteric lymph nodes, and thymus. Histologic changes in lymphoid tissue are similar to those observed in pups infected with CPV-2 but less severe. In addition, CPV-1 is capable of crossing the placenta and producing early fetal death and birth defects.[7] Experimental oronasal infection of neonatal specific pathogen-free (SPF) pups, with laboratory isolates from pups dying of enteric illness, produced only mild respiratory disease.[8] Naturally induced disease in young pups has been characterized by enteritis, pneumonia, and myocarditis.[47] Naturally infected dogs have been shown to have a reduction in both numbers and killing activity of phagocytes.[14]

Clinical Findings

CPV-1 has been observed infrequently in field dogs with mild diarrhea, as well as in the feces of clinically healthy animals. Primarily, CPV-1 infection is a cause of enteritis, pneumonitis, myocarditis, and lymphadenitis in pups between 5 and 21 days of age.[31] Many of these pups have mild or vague symptoms and eventually die, being classified as "fading pups." Affected pups usually have diarrhea, vomiting, and dyspnea and are constantly crying. Some puppies have respiratory disease with no enteric signs.[100] Sudden death with few premonitory signs has also been observed. Because of transplacental infections, this virus can cause failure to conceive or fetal death or abortion.

Diagnosis

CPV-1 infection should be considered in young (under 8-week-old) pups with mild diarrhea that clinically or histologically resemble CPV-2 disease but are serologically CPV-2 negative, or in unexplained fetal abnormalities, in abortions, or in fading pups. CPV-1 will not cross react with any of the serologic or fecal detection methods for CPV-2. EM has observed CPV-1 in fecal and rectal swab samples from field dogs. Immunoelectron microscopy is necessary to distinguish CPV-1 from CPV-2. Inhibition of hemagglutinating activity in stool suspensions by specific antiserum is also diagnostic for CPV-1. To determine exposure, sera can be tested for specific antibody with VN or HI tests. Because only the WRC cell line supports growth of CPV-1, the availability of virus isolation and serum VN tests is limited.

Pathologic Findings

Pathologic changes in nursing pups have included thymic edema and atrophy, enlarged lymph nodes, pasty soft stool in the intestinal tract, and pale gray streaks and irregular areas deep within the myocardium as found with CPV-2 (see Fig. 8-7). Histopathologic lesions are predominantly restricted to large intranuclear epithelial inclusions at the tips of the villi in the duodenum and jejunum. These inclusions are eosinophilic and often fill the nuclei. Other intestinal changes noted include crypt epithelial hyperplasia and single-cell necrosis of crypt epithelial cells. Lesions seen in other tissue include moderate to marked depletion or necrosis (or both) of lymphoid cells of Peyer's patches and thymus, severe pneumonitis with necrosis in airways, and mineralized focal to diffuse areas of myocardial necrosis with cellular infiltration.

Therapy and Prevention

Once a diagnosis has been made, treatment of pups suffering CPV-1 infection is unrewarding because of the rapid progression of the disease. However, mortality may be reduced by ensuring that the environmental temperature of newborn pups is kept warm and by adequate nutrition and hydration. No vaccine is available at present.

Public Health Considerations

No known public health concern exists; however, extra care should always be practiced in handling sick pups and fecal

material from diarrheic animals because other enteropathogens may be present.

CANINE CORONAVIRAL ENTERITIS

Etiology

CCV is a member of the virus family *Coronaviridae* belonging to the order *Nidovirales* (Fig. 8-8). Different coronaviruses of this family infect a large number of species, including humans, cattle, swine, dogs, cats, horses, poultry, rats, and mice (see Table 11-1). To date, several strains of CCV have been isolated from outbreaks of diarrheal disease in dogs. The virus genome is composed of a single-stranded RNA chain; replication occurs in the cell cytoplasm of the host. Coronaviruses are fairly resistant and can remain infectious for longer periods outdoors at frozen temperatures. The virus loses infectivity in feces after approximately 40 hours at room temperature (20° C) and 60 hours when refrigerated (4° C).[123] Coronaviruses can be inactivated by most commercial detergents and disinfectants.

Epidemiology

In 1971, a CCV was isolated from feces of military dogs that were suffering from suspected infectious enteritis. Since then, several outbreaks of contagious enteritis have occurred and a similar coronavirus has been isolated. The true importance of CCV as a cause of infectious enteritis in dogs is unknown; however, CCV was genetically detected[2] or isolated[70] from 16% or 57%, respectively, of dogs with diarrhea in Japan. Serologic testing of Australian dogs showing signs of diarrhea revealed that 85% were positive for CCV-IgM antibodies, which indicates recent infection.[70] Serologic information suggests that CCV has been present indefinitely in the dog population and is an infrequent cause of infectious enteritis. CCV is highly contagious and spreads rapidly through groups of susceptible dogs. Neonatal pups are more severely affected than those of weaning age and adult dogs. CCV is shed in the feces of infected dogs for weeks to months or longer, and fecal contamination of the environment is the primary source for its transmission via ingestion.[124]

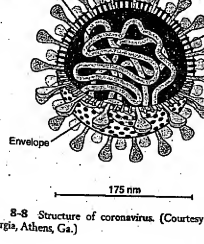

Fig 8-8 Structure of coronavirus. (Courtesy University of Georgia, Athens, Ga.)

Pathogenesis

The incubation period is short: 1 to 4 days in the field and only 24 to 48 hours experimentally. CCV can generally be isolated from the feces of infected dogs between 3 and approximately 14 days after infection.

After ingestion, CCV goes to the mature epithelial cells of the villi of the small intestine.[124,125] After uptake of CCV by M cells in the dome epithelium of Peyer's patches virus and viral antigen is transported to the underlying lymphoid tissue. Uptake in the gut lymphoid tissue suggests that CCV may persist or become latent in dogs, similar to the situation for feline coronavirus. Genetic analysis suggested that nucleotide substitutions occurred in the transmembrane protein M gene during the time of clinical illness.[104] The virus also rapidly reproduces within epithelial cells and accumulates within cytoplasmic vacuoles. Virions from these vacuoles may be released directly into the external environment via the apical plasmalemma or may be released after lysis of the apical cytoplasm of infected cells. After production of mature virus, infected cells develop severe cytoplasmic changes, and the microvilli of the brush border become short, distorted, and lost. The overall result is that infected cells become lost from the villi at an accelerated rate and are replaced by increased replication rate of immature cells in the crypts of the mucosa. Crypt epithelium is not destroyed; on the contrary, hyperplasia develops. Affected villi become covered by low columnar to cuboidal epithelium, show variable levels of villous atrophy and fusion, and become infiltrated by mononuclear cells in the lamina propria. Unlike CPV infection, villus necrosis and hemorrhage are rare.

Dogs can have CCV and CPV infections simultaneously, and some studies suggest that CCV infection enhances the severity of CPV infection. Conversely, three of four puppies in a litter died from CCV enteritis 2 weeks after surviving CPV enteritis.[107] Concurrent infections with canine adenovirus-1 and CCV was suspected as the cause of severe enteric disease in an animal shelter.[104] Other enteropathogens such as *Clostridium perfringens, Campylobacter* spp., *Helicobacter* spp., and *Salmonella* spp. may increase the severity of CCV illness (see Chapter 39).

Clinical Findings

Differentiating CCV from other infectious causes of enteritis is difficult. Theories suggest that CCV infection is usually less dramatic than CPV-2 infection. The clinical signs can vary greatly, and dogs of any breed, age, and sex are affected. This finding contrasts with CPV infections in which affected dogs are usually younger than 2 years. Infected dogs usually have a sudden onset of diarrhea preceded sometimes by vomiting. Feces are characteristically orange in color, very malodorous, and infrequently contain blood. Loss of appetite and lethargy are also common signs. Unlike CPV-2 infection, fever is not constant, and leukopenia is not a recognized feature.

In severe cases, diarrhea can become watery, and dehydration and electrolyte imbalances can follow. Concurrent ocular and nasal discharges have been noted, but their relationship to the primary infection is unknown. Most of the dogs affected recover spontaneously after 8 to 10 days. When secondary complicating factors are present (parasites, bacteria, or other viruses), the disease can be significantly prolonged.

Diagnosis

Making a definitive diagnosis of CCV-induced disease is difficult. EM can detect CCV in fresh feces. Approximately 1×10^6 virions are needed in unconcentrated fecal samples for identification of CCV by EM; thus false-negative findings are possible. Viral isolation is difficult because CCV does not grow well in tissue or cell culture systems. A highly sensitive reverse transcriptase PCR has been developed to detect CCV in fecal

specimens.[28,99,106] Serum VN and ELISA tests for CCV antibody have been developed.[111] Positive CCV serum titers of affected dogs can only confirm exposure to CCV, and serum IgG titers have no relationship to protection as do intestinal secretory IgA titers.

Pathologic Findings

Mild infections are grossly unremarkable. In severe cases, the intestinal loops are dilated and filled with thin, watery, green-yellow fecal material. Mesenteric lymph nodes are commonly enlarged and edematous.

Atrophy and fusion of intestinal villi and a deepening of the crypts characterize the intestinal lesions of CCV. Also present are an increase in cellularity of the lamina propria, flattening of surface epithelial cells, and discharge of goblet cells. With well-preserved tissues, FA staining can enable specific detection of virus in the intestinal lesions.

Therapy

Deaths associated with diarrheal disease are uncommon but occur in pups as a result of electrolyte and water loss with subsequent dehydration, acidosis, and shock. Management must emphasize supportive treatment to maintain fluid and electrolyte balance as described for CPV-2 infection. Although rarely indicated, broad-spectrum antimicrobial agents can be given to treat secondary bacterial infections. Good nursing care, including keeping the dogs quiet and warm, is certainly essential.

Prevention

Inactivated and MLV vaccines are available for protection against CCV infection.[23,87] Two doses 3 to 4 weeks apart and annual revaccination are recommended for immunization of dogs regardless of age. These vaccines are relatively safe but provide incomplete protection in that they reduce but do not completely eliminate replication of CCV in the intestinal tract after challenge.[86,89] Assessing the role of the CCV vaccines in protection against disease is difficult because CCV infections are usually inapparent or cause only mild signs of disease. For additional information on vaccination, see Coronaviral Infection in Chapter 100.

Public Health Considerations

CCV is not believed to infect people. Coronaviruses are not strictly host specific; thus the possibility of human infection cannot be excluded. For this reason, extra care should always be practiced in handling sick pups and fecal material from diarrheic animals.

CANINE ROTAVIRAL INFECTION

Etiology

Rotaviruses are recognized as important enteric pathogens in many animal species and in people. They are sometimes referred to as duovirus, reovirus-like, and rotalike virus agents. Currently, rotaviruses are classified as distinct members of the family *Reoviridae*. CRV is a double-stranded RNA, nonenveloped virus that is approximately 60 to 75 nm in diameter (Fig. 8-9). CRV is resistant to most environmental conditions outside the host.

Rotaviruses have been isolated in tissue cultures or observed by EM of specimens from many species, including mice, monkeys, calves, pigs, foals, lambs, humans, rabbits, deer, cats, and dogs.

Epidemiology

Rotaviruses are transmitted by fecal-oral contamination. The viruses are well adapted for survival outside the host and for passage through the upper GI tract. Serum antibodies to rotavirus have been identified in dogs and cats of all ages.

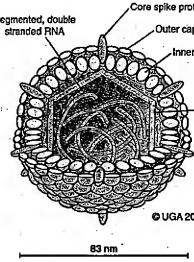

Fig 8-9 Structure of rotavirus. (Courtesy University of Georgia, Athens, Ga.)

Pathogenesis

Rotaviruses infect the most mature epithelial cells on the luminal tips of the small intestinal villi, leading to mild-to-moderate villous atrophy. Infected cells swell, degenerate, and desquamate into the intestinal lumen, where they release a large number of virions that become sources of infection for lower intestinal segments and for other animals. Necrosis of rotavirus-infected cells is most pronounced 18 to 48 hours after oral infection. Necrotic cells are rapidly replaced by immature crypt epithelium. Clinical signs result primarily from the villous atrophy, leading to mild to moderate maldigestion and malabsorption and osmotic diarrhea.

Clinical Findings

Most clinical rotaviral infections have been demonstrated in the feces of pups younger than 12 weeks, with mild diarrhea. Some cases of severe fatal enteritis associated with CRV have been reported to occur in pups as young as 2 weeks. The clinical signs are usually not as severe as those for the other canine enteric viruses (CPV-2 and CCV). A watery to mucoid diarrhea is usual, and this lasts for 8 to 10 days. The pups usually remain afebrile. CRV may contribute to enteric disease in mixed viral infections.

Diagnosis

Most pathogenic rotaviruses share common group-specific internal capsid antigens that can be detected by many methods, including commercial fecal ELISA (Rotazyme, Abbott Labs, N. Chicago, Ill.; Enzygnost, Behring Inst., Marburg, Germany) and latex agglutination (Rotalex, Orion Diag, Helsinki, Finland; Slidex Rota-kit, Biomerieux, Marcy-l'Etoile, France) tests used to diagnose human rotavirus infection (see also Appendix 6).[111] Rotaviruses can also be identified in fecal specimens by EM, although care must be taken to differentiate rotaviruses from the apparently nonpathogenic reoviruses occasionally present in dog feces. EM improves specificity of the test. Testing for seroconversion is possible but not widely available.

Pathologic Findings

Pathologic changes are limited to the small intestine, consisting of mild to moderate villous blunting. The virus can be detected in frozen sections by fluorescent antibody techniques.

Therapy and Prevention

Most dogs recover naturally from their infection. Treatment, if needed, consists solely of symptomatic therapy as described for CPV-2 enteritis. No vaccines are available for CRV, and current estimates of the frequency and severity of the disease do not appear to justify vaccine development.

Public Health Considerations

Rotaviruses are generally host specific; however, the various strains cannot be easily distinguished, and the possibility of human infection cannot be eliminated. Rotaviral infections in people usually occur in young infants and children (younger than 4 years). Poor sanitation and hygiene, as exist in developing countries, increase the prevalence of infection. Persons handling feces from diarrheic dogs should take routine precautions.

OTHER VIRAL ENTERITIDES

A large number of other viruses have been identified in feces of dogs both with and without diarrhea. For the most part, the pathogenicity and importance of these viruses as causes of infectious enteritis remain unknown. Based on work in other species, some viruses may be true enteric pathogens, whereas others are most likely incidental findings.

Astrovirus-like particles have been reported in the stools of clinically healthy and diarrheic dogs. Astroviruses are known to cause enteritis in other species, such as swine, but whether this is either true or common in the dog is unknown. The viruses have also been identified in diarrheic cats (see Chapter 12).

A herpesvirus antigenically related to feline herpesvirus has been isolated from a dog with diarrhea, but Koch's postulates have not been fulfilled.[31] Similarly, the importance of serologic reactivity of some dogs to human echoviruses and coxsackieviruses is unclear (see also Enteroviral Infections, Chapter 24).

An apparently specific canine calicivirus has been isolated on several occasions from the feces of dogs with enteritis, sometimes alone and sometimes in conjunction with other known enteric pathogens.[32,33] Similarly, an antigenically distinct parainfluenza virus, isolated from a dog with bloody diarrhea, was believed to be causal (see Chapter 7).

The study of viral enteritis in dogs is in its infancy. Undoubtedly, there are other viruses that affect the GI tract of dogs, but they remain to be discovered and characterized.

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*See the CD-ROM for a complete list of references.

CHAPTER • 9

Canine Viral Papillomatosis

Michelle Wall and Clay A. Calvert

ETIOLOGY

The papillomavirus was first described in 1933 when Shope discovered the agent responsible for cutaneous papillomas in the cottontail rabbit.[19] Multiple canine papillomas in dogs are uncommon, comprising less than 12.5% of canine skin tumors.[20] Benign mucocutaneous tumors of epithelial origin are caused by infectious papillomavirus of the *Papovaviridae* family. The papilloma viruses are categorized with the polyomavirus to form the papovaviruses. Members of this family are small (33 to 60 nm), naked, ether-resistant, double-stranded circular DNA tumor viruses, similar in structure to but larger than parvoviruses; they form crystalline structures within the nuclei of infected cells.[20,34] These viruses lack a lipid envelope and are acid stable and relatively thermostable, which may explain much of their inherent resistance.[34] Papillomaviruses are naturally oncogenic, producing benign warts, and are usually species and site specific. Cross-species infection of horses by bovine papillomaviruses type 1 and type 2 have been reported.[13] Most isolated viruses lack serologic cross-reactivity. Although antigenically distinct, papillomaviruses of humans, cattle, horses, dogs, and cats share at

APPENDIX C
Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix C contains Schwartz, D. *et al.*, *Virology 302*: 219-223 (2002) ("Schwartz *et al.*"). This publication was previously cited by the Examiner in a Notice of References Cited PTO-892 form accompanying the Office Action mailed on June 29, 2006.

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RAPID COMMUNICATION

The Canine Minute Virus (Minute Virus of Canines) Is a Distinct Parvovirus That Is Most Similar to Bovine Parvovirus

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We characterized the genome and proteins of the canine minute virus (the minute virus of canines (MVC)). The genome sequence showed MVC to be an autonomous parvovirus encoding a large nonstructural protein 1 gene, a smaller nonstructural protein, and overlapping VP1 and VP2 protein genes. The virus was most closely related to bovine parvovirus (BPV), with which it was 43% identical at the DNA sequence level, while the NS1 and VP1 proteins were 33.6 and 41.4% identical to those of BPV, respectively. Spliced messages of the NS1 gene transcripts were detected by RT-PCR. VP1 and VP2 proteins were detected in purified capsids, as were modified versions of each protein, and VP3 was also found in full capsids.

Introduction. The canine minute virus, also known as the minute virus of canines (MVC), was first described as an isolate from a healthy dog in the U.S.A. (*1*). That virus was grown in the Walter Reed canine (WRC) cell line and had the properties of a parvovirus, including small size (20–21 nm diameter), and the presence of virions in infected cell nuclei (*1–3*). The MVC appeared antigenically and genetically distinct from the canine parvovirus type-2 (CPV) based on antibody cross reactivity and restriction enzyme analysis of the viral DNA (*3*), but its genomic structure and relationship to other parvoviruses has not been described. The distribution of MVC and its association with canine diseases are also not well understood. Serological testing suggests that MVC is widespread in dogs in the U.S.A., with over 50% positive samples in the studies reported (*1, 4*). Although it is likely that most infections with MVC are subclinical, diseases associated with virus infection include fetal infections leading to reproductive failure and neonatal respiratory disease (*2, 5, 6*). The virus may also be associated with some cases of enteritis in puppies or older dogs (*1*). However, MVC is highly restricted in its tissue culture replication; there are few sensitive diagnostic tests widely available, and the true incidence of the virus infection or its associated diseases are therefore not known. Parvoviruses are widespread pathogens of vertebrate and invertebrate animals and are the cause of many different diseases. They replicate through a linear DNA replicative form (RF) and encapsidate a single-stranded DNA genome of either a single polarity, or from either DNA strand. Parvovirus capsids are assembled from between two and four overlapping capsid proteins that are generated by alternative splicing of one or two viral mRNAs, or by use of alternative start codons in one message (*7*).

Results. DNA sequence and analysis. The almost complete sequence of the genome of MVC was obtained from plasmid clones or directly from viral RF DNA and that covered 5097 bases (Fig. 1; GenBank Accession No. AF495467). We did not obtain the sequences of the very 3′ and 5′ ends of the genome, but by restriction analysis of the RF DNA with *Xho*I (nt 470 in the sequence determined) and *Bsa*MI (nt 5014 in the sequence determined), we determined that the sequence obtained extended to within 140 bases of the extended 3′ end and 150 bases of the extended 5′ end, suggesting a total genomic length of about 5390 bases. Electrophoretic analysis of the RF DNA digested with *Xho*I and *Bsa*MI showed that the turn-around and extended forms of the 3′ end differed in length by about 80 bases, and those of the 5′ end differed in length by about 75 bases, indicating that those palindromes contained about 160 and 150 bases, respectively (results not shown).

Comparing the MVC sequence with those of other parvoviruses showed that the most closely related virus

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0042-6822/02 $35.00

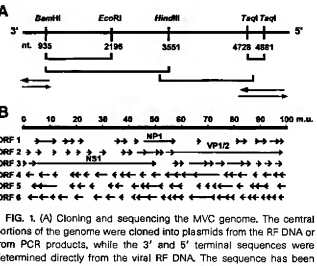

FIG. 1. (A) Cloning and sequencing the MVC genome. The central portions of the genome were cloned into plasmids from the RF DNA or from PCR products, while the 3′ and 5′ terminal sequences were determined directly from the viral RF DNA. The sequence has been deposited in GenBank (AF495467). (B) ORFs within the MVC sequence shown as arrows. Labels indicate the ORFs homologous with NS1, NP1, or VP1 and VP2 genes of the BPV.

was the bovine parvovirus (BPV), with which it shared 43% DNA sequence identity. When a conserved region within the translated sequence of the NS1 gene was compared to the equivalent sequence from other parvoviruses, MVC was again closely related to BPV, and the next closest relatives were the erythroviruses (human B19 and simian parvoviruses) (Fig. 2). MVC was quite distinct from canine parvovirus and feline panleukopenia virus and the other viruses in the rodent virus-related clade.

Large open reading frames (ORF) within the left and right halves of the genome encoded proteins homologous to the NS1 and the VP1 and VP2 proteins, respectively, of other parvoviruses, while a shorter ORF in the middle of the genome overlapped with the sequence of the NS1 protein, and that was similar to the predicted NP-1 protein of BPV (Figs. 1 and 3) (*8*). The NS1 sequence likely initiated at a methionine codon at nt 429 in the sequence and encoded a 717 residue protein with a predicted molecular weight of 81.9 kDa, which shared 32.6% identity with the NS protein of BPV (Fig. 3A). The right-hand ORF encoded VP1 and VP2 proteins of 78.9 and 63.9 kDa, respectively, which initiated at methionine codons in the same reading frame, at nts 2933 and 3329 in the sequence (Fig. 3B). The 21.7-kDa protein encoded by a third ORF was 39% identical to the NP-1 protein predicted from the BPV sequence (Fig. 3C) (*8*). The first 188 nts of the NP1 ORF overlapped with the C-terminal sequence of NS1.

Analysis of mRNAs by RT-PCR. We used RT-PCR to isolate spliced mRNA products from MVC-infected cells and then cloned the products and examined their sequences. Two different pairs of PCR primers were used which would flank the introns described for many parvoviruses (Fig. 4). For the RT-PCR spanning the region around the beginning of the VP1 ORF between nts 2601 and 3346 (Fig. 4, primer pair 2 and 3), the only product detected was the intact 745-bp sequence, and no clones were identified that would represent splices of these sequences. For the RT-PCR spanning the 368- to 3346-nt region of the genome (primer pair 1 and 3 in Fig. 4), the predicted full-sized 2978-nt product was detected, as well as products that appeared to be about 1100 nts and smaller than 400 nts (Fig. 4). Clones isolated from the middle-sized product had two splices that fused two sequences within NS1 and part of the NP1 ORF. Smaller products showed at least two sequences—in one 26 residues from the N-terminus of NS1 were fused to the VP1 ORF, while the other fused the NS1 ORF to an alternative ORF overlapping that of NP1. Whether the NS1–VP1 fusion is formed in any quantity was not determined, but it is possible that those represent the origin of the alternative forms of VP1 recovered from the viral capsids (Fig. 5).

Protein Analysis. Purified MVC capsids contained a number of protein forms, with a minor and major form representing the VP1 and VP2 proteins (Fig. 5). The major form of VP1 had an estimated size of 81 kDa, while the VP2 forms were 67 and 63 kDa. VP3 (61 kDa) was present in the full virus capsids, likely resulting from cleavage of VP2 at arginine 19 in the VP2 sequence, similar to that reported for MVM and CPV (*9*). Western blotting of the proteins from MVC-infected cells using a

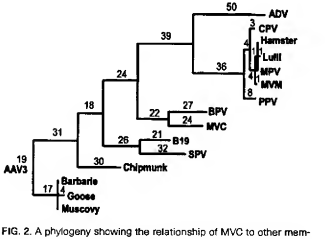

FIG. 2. A phylogeny showing the relationship of MVC to other members of the family *Parvovirinae*, including Aleutian mink disease virus (ADV), canine parvovirus (CPV), hamster parvovirus (hamster), LuIII, mouse parvovirus (MPV), minute virus of mice (MVM), porcine parvovirus (PPV), BPV, MVC, human parvovirus B19 (B19), simian parvovirus (SPV), chipmunk parvovirus (chipmunk), barbarie duck parvovirus (barbane), goose parvovirus (GPV), and Muscovy duck parvovovirus (Muscovy). The tree was rooted using adenoassociated virus (AAV) as an outgroup. A conserved sequence of 149 residues of the MVC NS1 was aligned with the sequences of the other viruses indicated and the most parsimonious phylogeny determined using the program PAUP 3.1.1. The single tree determined is shown; the numbers shown indicate the number of residues on each branch.

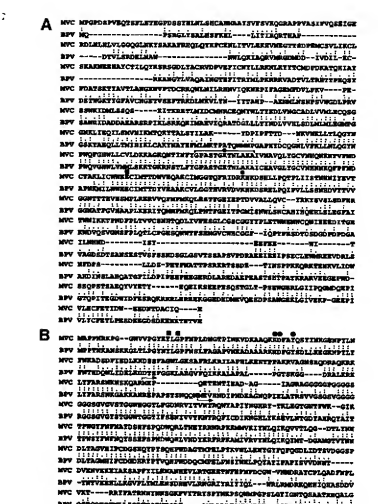

FIG. 3. Alignments of the MVC protein sequences (top) with those of BPV (bottom). (A) The NS1 ORF. Within the conserved central region of the protein, the nucleotide-binding site required for DNA nicking identified in the MVM NS1 sequence is marked by the overlining. Conserved residues that in MVM have been associated with nucleotide binding or ATPase activity are indicated by circles and squares, respectively. (B) The VP1/VP2 ORF. The conserved phospholipase A2-specific residues in that sequence are indicated. Closed circles indicate the active site motif (HDXXY) and the closed squares indicate the Ca^{2+} binding loop residues (YXGXG). The arrow indicates the predicted start of the VP2 sequence. (C) The NP-1 ORF.

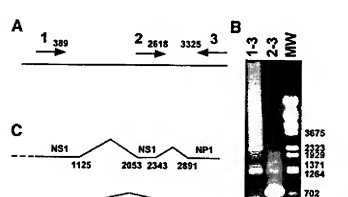

FIG. 4. The RT-PCR analysis of viral mRNA transcripts. (A) The RT-PCR reactions performed are diagrammed, showing the three primers that were used to amplify the messages. Numbers indicate the nucleotide in the genome complementary to the 3′ end of each primer. (B) The RT-PCR products detected in an agarose gel. Size of the molecular weight standards in base pairs. (C) Spliced forms identified in the PCR reactions, as determined from products cloned into plasmids and sequenced. The nucleotides at the junctions of the different products are given.

postinfection dog serum showed the VP2 protein, as well as a protein band of about 80 kDa which could represent the VP1 and NS1 proteins, which are close to this size and would likely be recognized by the postinfection serum (Fig. 5B).

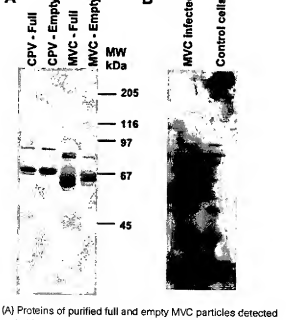

FIG. 5. (A) Proteins of purified full and empty MVC particles detected by Coomassie blue staining, compared to those of CPV full and empty capsids. Size standards are shown in kDa. (B) Proteins from infected or uninfected WR cells electrophoresed on a 10% acrylamide gel, transferred to a nitrocellulose membrane, and then probed with antibodies from an SPF dog that had been infected with MVC.

Discussion. The MVC has been associated with several different diseases of fetal and neonatal puppies, and by serological testing the virus appears to be widespread in dogs, most likely causing subclinical infections in many infected animals (*1–6, 10*). Here we show that MVC is a distinct member of the *Parvoviridae* which is most closely related to the bovine parvovirus, although it shares only 43% identity in DNA sequence with that virus. MVC was only distantly related to the well-characterized CPV, as has been suggested by serological testing and by restriction enzyme analysis of the viral DNA (*3*).

The genetic structure of MVC was typical of a parvovirus, with large ORFs encoding the NS1, VP1, and VP2 proteins, along with a small ORF that partially overlapped with the NS1 gene that was equivalent to the NP1 protein of BPV (*8, 11*). The function of the NP1 protein is unknown, and there is no obvious homology with any protein in the data bases. The NS1 protein sequence had a conserved core that contained the sequences identified as being involved in the nucleotide binding and DNA-nicking activities of the protein (*12, 13*) (Fig. 3A). Sequences identified in the MVM NS1 as a metal coordination site (histidines 127 and 129), and to be part of the active site (tyrosine 210), could not be clearly identified in the NS1 of MVC or BPV. Conserved sequences that are associated with the active site of a phospholipase A2 activity of the VP1 unique region of several parvoviruses were present in the MVC and BPV sequences, suggesting that that was a highly conserved function of the protein (Fig. 3B) (*14*).

The mRNA transcripts identified by RT-PCR between primer sites around nts 390 or 2618 and 3325 showed a variety of possible mRNA forms producing a variety of proteins (Fig. 4). We cannot define the significance of those spliced products, but they appear similar to those described for the Aleutian mink disease virus and the human parvovirus B19, where a variety of spliced products have been reported, but for most of which the functions have not been defined (*15–17*). The two capsid proteins appear similar to the VP1 and VP2 proteins of the other parvoviruses, but no evidence was found of a spliced product within the 2618- to 3325-nt region, and the two proteins are likely encoded by a single message with translation initiating at two ATG codons to give VP1 and VP2.

The evolution and relationships of the parvoviruses is only partially understood (*18, 19*). Among the viruses of vertebrates there appear to be at least three distinct clades, which include adenoassociated viruses which are more closely related to the avian-derived viruses, the rodent virus-related viruses which include canine parvovirus and minute virus of mice, and the erythroviruses which include B19 and similar viruses from primates, as well as the more distantly related BPV which is now grouped with MVC. However, MVC is still only distantly related to the other viruses, indicating that it diverged in the distant past.

Materials and Methods. Virus growth, capsid, and DNA purification. The GA3 isolate of MVC (*5*) was grown in thinly seeded Walter Reed canine cells (*1*). Replicative form DNA was harvested from infected cells 2 days after virus inoculation by modification of the method of Hirt (*20*). The cells were lysed into 10 mM Tris–HCl (pH 7.4) 10 mM EDTA, 0.6% SDS; then 20 mg/ml of Pronase was added and incubated for 2 h at 37°C. The lysate was made up to 1 M NaCl, incubated on ice, and then centrifuged at 80,000 *g* for 1 h. The supernatant was collected, ethanol precipitated, made up into 50 mM Tris–HCl (pH 7.5), 5 mM EDTA, and extracted with phenol and then with chloroform. The RF DNA was purified from a 1% agarose gel by spin column extraction.

To prepare virus for protein analysis, WRC cells were inoculated with virus for 1 h at 37°C. After 4 days of incubation, the cells were frozen and thawed twice. Capsids were pelleted from the culture supernatant at 80,000 *g* for 3 h; the pellet was sonicated for 2 min, and then the virus was banded in 10 to 40% sucrose gradients at 90,000 *g* for 6 h. Proteins in each gradient fraction were electrophoresed in 10% SDS–polyacrylamide gels (*21*), and the gels stained with Coomassie blue. Purified full and empty capsids of canine parvovirus were included for comparison. To examine the proteins in infected cells, lysates of infected cells were electrophoresed in 10% PAGE and then transferred to nitrocellulose membranes and incubated with a postinfection dog serum. The blot was incubated with goat anti-dog IgG conjugated with horseradish peroxidase and then with Super-Signal substrate (Pierce Chemical, Rockford, IL).

DNA Cloning and Sequencing. The strategy for cloning and sequencing the viral genome is diagrammed in Fig. 1A. The RF DNA was digested with *Bam*HI, *Eco*RI, *Hin*dIII, or *Taq*I (*3*). DNA fragments were cloned into the vector pGEM3Z (Promega, Madison, WI); sequences were obtained with SP6 and T7 primers from the flanking regions in the plasmids. Sequences were determined in both orientations by automated sequencing with a variety of specific primers. A 1177-base region between the *Hin*dIII site and a *Taq*I fragment was amplified from virus DNA by PCR and cloned into the vector pGEM-T easy (Promega), and three clones sequenced. To obtain sequences near the ends of the genome, purified viral RF DNA (100 ng/μl) recovered from infected cells was sequenced directly using specific primers annealing near the ends of the sequences obtained from the cloned DNA fragments, and sequences were also obtained directly from the RF DNA using reverse primers. The viral genomic sequence was analyzed using the program DNAStar. The translated open ORFs identified were aligned with those of BPV, the most closely related sequence in GenBank.

A highly conserved region of the NS1 gene (residues 332 to 481, equivalent to residues 412 and 562 of the MVMp NS1 sequence) was aligned with the homologous regions of other parvoviruses, and the phylogenetic relationships were determined using the branch-and-bound algorithm of the program PAUP 3.1.1.

RT-PCR and Sequencing of Viral Messages. RT-PCR was used to examine for possible spliced products in the viral RNAs. Primers prepared were from positions 368–389 (5′-GCAAAGAGCACTGGGCGGTATT-3′), 2601–2618 (5′-ACCTCTTCCTGCGTTCTG-3′), and 3346–3325 (5′-AGTTTCCTGCGGTTCCATCCTA-3′). RNA was isolated using the RNAgents kit (Promega); then cDNA was prepared from the total RNA using specific primers and the Access RT-PCR kit (Promega). The PCR products were run on a 1% agarose gel. To determine the origins of specific products, the amplified DNAs in different bands were isolated from the agarose gel and ligated into the pGEM-T Easy plasmid. Then plasmids were sequenced using the T7 and SP6 primers in the vector.

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APPENDIX D

Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix D contains Pratelli *et al., J. Vet. Diag. Invest. 11*: 365-7 (1999).

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Functional consequences of cor triatriatum dexter in dogs is dependent on presence or absence and the size of the opening in the septum between the two compartments.[7] The main historical finding in this case and most previously reported cases is prolonged abdominal distention due to ascites, ranging from 6 weeks to 2 years in duration.[4–8,11–13] Ascites was not the main clinical feature in 7-year-old German short-haired pointer that presented with exercise intolerance and episodic weakness of over >3 years' duration[12] or in a 6-year-old German shepherd dog cross that presented with a 24-hour history of lethargy.[8] Age at diagnosis ranged from 8 weeks to 7 years, and breeds included chow chow,[3,4,7] cocker spaniel,[8,12] English bulldog,[4] German shepherd dog cross,[1] German short-haired pointer,[12] golden retriever,[13] greyhound,[4] rottweiler,[5,6] and mixed breed.[4,11] Another common finding in previous cases is hepatic congestion and/or hepatomegaly.[6,7,13] Venous engorgement and distention were noted in the abdominal veins and in the body wall in previous cases.[7,11–13] Where ascitic fluid was evaluated, there were high-protein (3.4–5.8 g/dl) modified transudates with specific gravities ranging from 1.015 to 1.028.[4,6,7,12,13]

Cor triatriatum dexter is an extremely uncommon congenital heart defect in dogs. This is the ninth report of canine cor triatriatum dexter, and it represents a third unique anatomic variant, which is characterized by partitioning of the right atrium by a perforate diaphragm or septum with the coronary sinus and fossa ovalis located in the cranial chamber. Cor triatriatum dexter represents a diagnostic challenge at necropsy, requiring careful examination of the heart. This defect should be considered as a differential diagnosis in young dogs with signs of abdominal distention and ascites.

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J Vet Diagn Invest 11:365–367 (1999)

Fatal canine parvovirus type-1 infection in pups from Italy

Annamaria Pratelli, Domenico Buonavoglia, Maria Tempesta, Franco Guarda, Leland Carmichael, Canio Buonavoglia

Canine parvovirus type 1 (CPV-1), also known as minute virus of canines (MVC), is an autonomous parvovirus of dogs that was isolated in 1967 from normal canine feces.[1] Antigenic and genomic properties of MVC are distinct from those of canine parvovirus type 2 (CPV-2), which emerged in 1978 in the canine population and is responsible for worldwide outbreaks of severe hemorrhagic gastroenteritis in dogs.[2,3]

The natural pathogenicity of MVC for dogs is undetermined; however, the virus has been isolated from the feces of normal dogs[1] and of dogs with mild diarrhea[4] and from the small intestine and lungs of young pups with mild to fatal enteritis.[4–9] Experimental studies have shown that CPV-1 may cause mild to severe pneumonitis and enteritis in neonatal pups and embryo resorptions or fetal deaths in pregnant bitches infected between gestational days 25 and 35.[5,6] In this report, we describe a natural outbreak in Puglia (Italy) of fatal CPV-1 infection in 35-day-old pups with pulmonary and cardiac disease.

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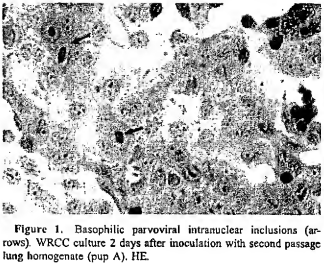

Figure 1. Basophilic parvoviral intranuclear inclusions (arrows). WRCC culture 2 days after inoculation with second passage lung homogenate (pup A). HE.

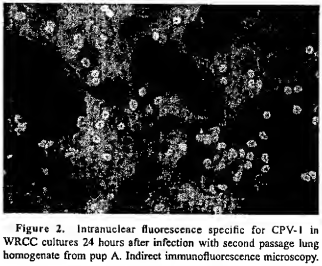

Figure 2. Intranuclear fluorescence specific for CPV-1 in WRCC cultures 24 hours after infection with second passage lung homogenate from pup A. Indirect immunofluorescence microscopy.

Two 35-day-old German shepherd pups from a litter of 5 died suddenly and unexpectedly after a brief period of respiratory distress. The other 3 pups in the litter had mild respiratory symptoms, but they recovered within 7 days. Enteric signs were not noted. Macroscopic findings in 2 dead pups were severe pneumonia and occasional pale areas in the heart muscle, suggestive of myocarditis. The intestinal tract was grossly normal.

Samples of lung and heart from the 2 dead pups (A and B) were homogenized, and 20% suspensions (w/v) were made in RPMI medium[a] containing antibiotics (200 IU/ml penicillin, 200 mg/ml streptomycin, 50 units/ml amphotericin B). After low-speed centrifugation, 0.2-ml portions of the supernatant of each sample were inoculated onto Walter Reed canine cell (WRCC) cultures in 25-cm² plastic tissue culture flasks[b] immediately after trypsinization. The cells were grown in RPMI medium containing 10% of bovine fetal serum. Tissue samples were also inoculated onto WRCC cultures grown in tissue culture chamber slides[b] and incubated at 37 C in a 5% CO_2 incubator.

Cell cultures were observed daily for the appearance of cytopathic effects (CPE). After 8 days of incubation, in the absence of CPE, the cell cultures were frozen and thawed 3 times and the cryolysates were used for subsequent passage.

The indirect immunofluorescent assay (IFA) employed a specific-pathogen-free (SPF) canine serum positive for CPV-1 and a fluorescein-labeled anti-dog IgG.[c] Tests for viral antigen were carried out on frozen tissue sections of lung and heart from the infected pups and on control tissues from normal pups. Inoculated and normal WRCC multichamber cultures were examined by IFA.

The IFA test also was used to measure CPV-1 antibody responses of the bitch and the recovered pups. The test employed acetone-fixed WRCC in multichamber culture slides infected with CPV-1 strain GA3.[c] For testing, fixed cells were emersed briefly in phosphate-buffered saline (PBS), pH 7.2. Two-fold dilutions of serum from the bitch or recovered pups were then added in 0.2-ml amounts to duplicate chambers and allowed to stand for 30 minutes. After rinsing 3 times in PBS, the slides were treated with the labeled anti-dog IgG and, after 30 minutes, were examined with a fluorescence microscope. The antibody titer was considered the highest dilution of serum that gave specific fluorescence. Controls comprised a canine anti-CPV-1 serum with an IFA titer of 1:320 and a normal SPF canine serum.

Tissue samples were obtained from the lungs and hearts of the dead pups and fixed in 10% buffered formalin. Fixed tissues for histologic examination were cut in 5-μm sections, mounted on glass slides, and stained with hematoxylin and eosin (HE).

The IFA test revealed specific fluorescence in frozen lung sections from pups A and B; however, the fluorescence was weak and was present only in limited areas of the sections, especially those from pup B. Specific fluorescence was not observed with normal pup lung tissues.

Following the inoculation of WRCC cultures with the tissue homogenates, mild CPE was observed on the first passage only with samples from pup A. Uninoculated cell cultures remained normal. At the second passage, however, CPE was evident in WRCC cultures inoculated with samples from both pups. The CPE induced by virus isolated from pup A (strain C/9871) appeared earlier and was more evident in subsequent passages. Basophilic intranuclear inclusion bodies, typical of parvovirus infection, were observed in the inoculated cell cultures after HE staining (Fig. 1). Intense intranuclear fluorescence was present in WRCC chamber cultures 24 hours postinoculation (PI) with second-passage lung culture cryolysates from pup A (Fig. 2). Specific fluorescence was noted in chamber cultures inoculated with the second passage tissue samples from pup B, but the fluorescence was present only in scattered cell nuclei.

The prominent histologic lesion in the lungs was interstitial pneumonia with accumulations of mononuclear cells, neutrophils, and rare macrophages in lesion areas (Fig. 3). Alveoli contained edema fluid and variable numbers of granulocytes. Nuclear degeneration with karyolysis and pyknosis and, less frequently, karyorrhexis were evident in alveolar epithelial cells. A small number of basophilic intranuclear inclusion bodies was observed in scattered alveolar cell nuclei (Fig. 4). The myocardium had mild focal degenerative changes, but intranuclear inclusion bodies were not observed and specific flourescence was not seen after IFA staining.

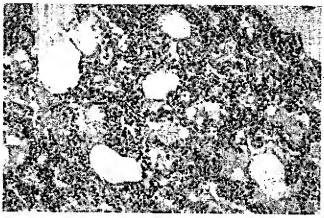

Figure 3. Interstitial pneumonitis with alveolar edema (pup A). HE.

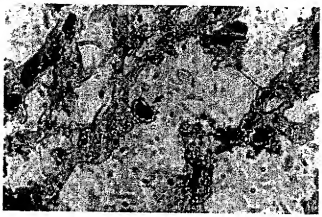

Figure 4. Intranuclear inclusion (arrow) in frozen section of lung tissue (pup A). HE.

Additional evidence of CPV-1 infection was the presence of high levels of antibodies to CPV-1 by the IFA test. The bitch had a titer of 1:160, and the 3 surviving pups had titers ≥1:640.

The natural pathogenicity of CPV-1 for dogs appears to be highly variable. This virus has been isolated from healthy dogs[1–3] and from pups that died with signs of enteric and respiratory illness and, in some cases, myocarditis.[4–8]

In this outbreak of natural CPV-1 infection in Italian dogs, pneumonitis was the principal finding at necropsy. Detailed pathologic findings could not be reported because of the condition of tissues received, but changes observed in the lungs were similar to those reported in other natural and in experimental CPV-1 infections.[4,7] Unfortunately, intestinal samples were not saved because of the lack of reported enteric signs or gross lesions.

The case reported here is epidemiologically significant because it confirms the presence of CPV-1 in Italy. Also, the presence of respiratory, but not enteric, illness in pups >1 month of age is of interest because previous cases have occurred in pups <3 weeks of age and enteric signs were most prominent. Because antibodies to CPV-1 (MVC) appear to be common in the dog population in the United States and, in limited studies, in Europe and Japan,[1,2,6] MVC should be considered when young pups die suddenly with signs of respiratory or enteric disease or in cases of sudden death with heart failure. Transplacental infections that resulted in fetal deaths, mummification, and early abortions also have been documented experimentally.[3] Studies are underway to determine the seroprevalence of CPV-1 in Italy and to further assess the role of this virus in reproductive failures and young pup mortality.

Sources and manufacturers

a. GIBCO Laboratories, Grand Island, NY.
b. Nunc, Naperville, IL.
c. Sigma Chemicals, St. Louis, MO.

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APPENDIX E

Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix E contains Truyen, U., Recent Advances in Canine Infectious Diseases, International Veterinary Information Service (January 2000). This publication was cited by the Examiner in the PTO-892 form accompanying the Office Action mailed on March 21, 2007.

IVIS

In: **Recent Advances in Canine Infectious Diseases**, L.E. Carmichael (Ed.)
Publisher: International Veterinary Information Service (www.ivis.org)

Canine Parvovirus (26 Jan 2000)

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Classificaton and Epidemiology

Two distinct parvoviruses (CPV), are now known to infect dogs - the pathogenic CPV-2, which was recognized as a new disease of dogs and wild canines in 1978, and the "minute virus of canines" (MVC, CPV-1) reported by Binn in 1970. MVC, a completely different parvovirus, had not been associated with natural disease until 1992. MVC may cause pneumonia, myocarditis and enteritis in young pups, or transplacental infections in pregnant dams, with embryo resorptions and fetal death. Confirmed infections have been reported in the USA, Sweden, Germany, and, more recently in Italy. Only about 30 cases have been reported.

Canine parvovirus (CPV, CPV-2) and feline panleukopenia virus (FPV) are very closely related and are important pathogens of their respective hosts, the dog and cat. CPV-2 infects dogs and other Canidae such as wolves, coyotes, South American dogs and Asiatic raccoon dogs, but not cats. FPV and the FPV-like viruses infect both large and small cats, as well as mink, raccoons, and possibly foxes, but not dogs. However, the clear separation of a cat virus infecting only cats (FPV) and a dog virus infecting only dogs (CPV-2) is no longer certain as the original dog virus, CPV-2 was transitory, and replaced in nature by so-called 'new antigenic types' (CPV-2a and CPV-2b) that infect or replicate in, and are transmitted between, dogs and cats. FPV has long been known as the cause of disease in cats, raccoons and certain related carnivores, but CPV is a genuine newly emerged virus which was probably derived from a close relative of FPV during the 1970s and has, since 1978, established itself in dog populations throughout the world. Amino acid (AA) sequences which comprise the surface proteins of the viral capsid are the primary determinants of the host range of parvoviruses, and only a few AA differences between CPV and FPV determine the ability of each virus to replicate in dogs, cats or their cultured cells. Although CPV and FPV isolates are >98% identical in their DNA sequences, the viruses can be readily distinguished by antigenic typing with monoclonal antibodies. The determinants of host range differences among the various parvoviruses are complex. All members of the group comprising CPV/FPV replicate in feline cells in tissue culture, but only isolates from dogs replicate in cultured canine cells. Their in vivo host ranges also differ, since FPV isolates replicate efficiently only in cats, whereas CPV isolates show variable replication in cats or feline cell cultures, depending on the strain of CPV. The original CPV-2 isolates do not replicate in cats, but the variants of CPV-2, designated CPV-2a and CPV-2b, replicate efficiently in cats. In addition, CPV-2a and CPV-2b have been isolated from cats in Japan, Germany and the USA which had natural parvovirus disease indistinguishable from panleukopenia.

CPV appears to have been present initially in Europe and it subsequently spread throughout the world in 1978-1979 in a period of about 6 months. As noted, the origin of the original virus is not known, although it most likely was derived from a closely related virus of other carnivore species - cats, mink, raccoons, Asiatic raccoon dogs, or foxes. It was then replaced between 1979 and 1984 by the two distinct antigenic variants. Derivation from an FPV vaccine strain in tissue culture was suggested as one possibility, but subsequent studies did not reveal any support for that hypothesis, and derivation from a virus in nature, e.g., a wild carnivore such as the European red fox (Vulpes vulpes) appears more likely.

The extension of the in vivo host range to cats and dogs has important epidemiological consequences. Any dog with parvovirus infection also is a potential carrier of the virus to susceptible (non-vaccinated) cats.

Parvovirus disease in cats is mainly caused by FPV, however CPV-2a or -2b viruses have been isolated from approximately 5% of samples submitted for feline panleukopenia diagnosis, indicating that some parvovirus infected cats may also transmit CPV to susceptible dogs. This finding should be recognized by veterinarians who treat both cats and dogs. Another unexpected finding from retrospective studies of tissues from large cats, e.g., cheetahs and tigers, was the occurrence of parvoviral disease in zoos in the USA, Southern Africa and Germany, where it was found that the cats were diagnosed as infected with CPV-2a or -2b; only 30% were found infected by FPV. This may indicate a higher susceptibility of large cats to CPV, a situation which is similar to that with canine distemper virus, which also has been shown to be the cause serious or fatal infections of large cats.

Pathogenesis

CPV replicates in several lymphoid tissues and the intestinal epithelium of dogs, and FPV replicates in the corresponding tissues in the cat; however, there are differences in the extent of viral growth in tissues of the two species. The pathogenesis of infections by CPV and FPV in dogs and cats are very similar. The route of entry and initial sites of virus replication are cells of the nasal- and oral-pharynx, including the tonsils and other lymphoid tissues. Animals can be experimentally infected by most parenteral routes; however, the oral route is the most common natural route of infection. Virus spreads systemically via a viremia, and it is found after 1 - 3 days in the tonsils, retropharyngeal lymph nodes, thymus and mesenteric lymph nodes. By 3 days post-infection, virus also can be recovered from the intestinal-associated lymphoid tissues (Peyer's patches). It is important to recognize that infection of the crypt cells of the intestinal epithelium occurs after the viremic phase, and it is not derived directly from ingested virus in the gut lumen. Circulating neutralizing antibodies, therefore, are able to minimize the extent of infection of the intestinal epithelium, but they do not prevent infection unless antibody levels are high. This phenomenon has relevance to vaccination since inactivated vaccines may prevent disease for several months, but they do not prevent actual infection, except for a few post-vaccination weeks. Cytokines may play an important role in the pathogenesis of CPV/FPV infections, however studies have not been reported.

Clinical Signs and Pathology

Clinical signs of CPV are well known, and only briefly reviewed here since they have been reviewed in several publications. Disease is often asymptomatic in older dogs or in pups that receive a low virus dose since the severity of infection is highly dose related. For example, a pup may acquire infection by CPV in a contaminated kennel, dog show, or veterinary clinic and experience only mild, or no illness. However, virus amplified in the intestine of that pup would be shed in large amounts to littermates or other susceptible dogs in contact. In contrast to the marked panleukopenia seen in cats infected with FPV, a relative lymphopenia, not panleukopenia, is often observed in dogs infected with CPV. Lymphocyte numbers decline, but there is little effect on eosinophil, basophil, monocyte, or red cell numbers. Interestingly, in experimental studies of cats with a CPV-2b isolate, the virus caused only a slight leukopenia but there was a marked lymphopenia, similar to the pattern seen in CPV infection of dogs. Infection of the lymphoid tissues with CPV results in lymphocytolysis, cellular depletion and, subsequently, tissue regeneration in surviving animals. Virus replication and cell destruction in lymphoid tissues occur primarily in areas of dividing cells, including the germinal centers of lymph nodes (Fig. 1) and the thymus cortex. In clinically infected dogs, dehydration is severe and early treatment with electrolyte solutions is essential. Profound weight loss also is characteristic of CPV infection and the architecture of the small intestine is not restored to normal for 2 - 3 post-infection weeks, at which time weight losses return to normal. Certain breeds, e.g., Doberman Pinschers, Rottweilers and English Springer Spaniels are reported to be at higher risk of severe disease. Parvovirus disease may be exacerbated by concurrent infections with Giardia, hookworms, other enteric organisms or canine coronavirus.

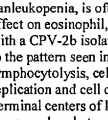

Figure 1. CPV viral antigen in mesenteric lymph node 4 days after oral-nasal infection (Immunofluorescence microscopy).To view this image in full size go to the IVIS website at www.ivis.org .

The mode of intestinal infection appears to be similar with both feline and canine parvoviruses. FPV and CPV infect the rapidly dividing epithelial cells in the crypts of the intestinal villi of the ileum and jejunum between

3 and 5 days after infection (Fig. 2). The degree and the severity of the infection are in part determined by the rate of turn-over of the intestinal epithelial cells. The severity of clinical disease probably reflects the extent of damage the virus produces to the small intestine. During the intestinal phase of the infection, virus is excreted in large amounts in the feces (Fig. 3). Virus is commonly shed from post-infection days 3 - 9, with peak titers occurring at the time, or prior to, the onset of clinical signs. It is important to note that a carrier state has not been demonstrated.

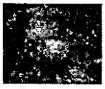

Figure 2. CPV viral antigen primarily in crypt areas of small intestinal (ilium) epithelium (Immunofluorescence microscopy).To view this image in full size go to the IVIS website at www.ivis.org .

Figure 3. Parvoviral particles in the feces of infected dog. Field casse. (Electron photomicrograph x30,000).To view this image in full size go to the IVIS website at www.ivis.org.

Infection of neonatal pups, unlike that in older animals, is characterized by infection of the developing heart in puppies (Fig. 4). In contrast to the infection of pups, kittens which become infected either in utero, or shortly after birth, experience viral replication in cells of the external germinal epithelium of the cerebellum, resulting in cerebella hypoplasia. CPV infection of neonatal pups can result in death from myocarditis, generally between 3 - 8 weeks of age, but deaths may occur up to 16 weeks of age or, rarely, longer. The age-dependence of the myocardial and cerebellar infections of cats is due to the active cell division in those tissues, but only in very young animals. Neither myocarditis in kittens, nor cerebellar lesions in puppies, have been reported with parvovirus infections. Neonatal infections also can result in generalized infection with lesions in several tissues. Unlike CPV infection in dogs, in utero infections of cats with FPV or Arctic foxes with blue fox FPV, may result in fetal death and resorption, abortion or neonatal death.

Figure 4. Heart from puppy which died of parvoviral myocarditis. Note the necrotic (light) areas in myocardium..To view this image in full size go to the IVIS website at www.ivis.org.

Diagnosis

Several laboratory tests have been developed and are available for specific viral diagnosis. Where facilities are available, rapid diagnosis can be made by electron microscopy (EM) of fecal material from cases with typical signs of disease. The virus also can be isolated in several feline and canine cell lines such as canine and feline kidney cells, but isolation is seldom used in practice since cell cultures are required and at least 1 week for results is required. Fecal hemagglutination-hemagglutination inhibition (HA-HI) tests have provided a simple and rapid method for detecting virus in fecal and tissue samples and are employed by several diagnostic laboratories in the USA, however the HA test is less sensitive than EM or enzyme-linked immunoassays (ELISA) . Several species' erythrocytes, e.g., pig, rhesus monkey or cat red cells, have been used. For specificity, a second HA test is done, using 10% fecal extracts from suspect cases, but with prior addition of specific immune serum, or monoclonal antibodies, to the extracts. Tests based on ELISA are commercially available and are based on an antigen-antibody reactions with specific monoclonal antibodies fixed on plastic, nitrocellulose membranes, latex or gold particles. The tests are rapid and relatively cheap, and can be

performed in any veterinary practice or clinic. The specificity of the tests relate to the antibodies used in the test. A problem in the past was an unacceptably high percentage of false positive results. Stringent quality control of each antigen lot appears critical. In general, about 103 particles per gram feces can be detected by electron microscopy or ELISA. Serological tests have limited value for diagnosis since high titers are usually present at the onset of clinical illness. However ELISA tests may detect specific IgM antibodies, which occur early in infection, but disappear after 2 - 3 weeks post-infection. Recently a semi-quantitative "Immunocomb Test" (Galed BioLabs, Israel) has been developed and has been made available commercially. That test can be performed by clinics or diagnostic laboratories and detects antibodies to CPV, where titers are reported to correlate well with HI test results. About 10-fold higher sensitivity can be achieved by using the polymerase chain reaction (PCR), but this assay is available only in a few laboratories and has been mainly used for research.

Therapy

The restoration of the electrolyte and fluid balance is the most important goal of therapy. Antibiotic treatment to reduce or prevent secondary bacterial infections are recommended. During the early phase of the disease the application of hyper-immune serum may help to reduce the virus load and render the infection less dramatic. Such treatment, used experimentally, has been shown to reduce mortality and shorten the length of the disease; however, the hyper-immune sera is difficult to obtain.

Immunization

Effective vaccines are available for the prevention of CPV-2 infection. Both modified live and inactivated parvovirus vaccines have been shown to immunize fully susceptible (seronegative) pups. Attenuated strains of CPV have been derived by repeated passage of the viruses in cell culture. The attenuating mutations in those viruses are not known, but the vaccine viruses are shed at much lower titers in the feces, suggesting that the absence of enteritis results from decreased viral replication in the intestine. Experimentally, live virus vaccines have been shown to protect for at least 3 years, or longer. Inactivated vaccines, however, provide only a limited duration of immunity to infection, although dogs may be protected against disease for several months. For parvovirus prophylaxis, MLV vaccines have proved to be much more effective than inactivated vaccines. This has led to the virtual removal of inactivated vaccines from the German market MLV vaccines have been shown to be safe, and neither vaccine-induced diseases, reversion to virulence or the involvement of vaccine viruses in the generation of 'new viruses' have been confirmed.

There is a strong correlation between HI or serum neutralizing (SN) antibody titers and resistance to infection with CPV. The HI test has been useful to measure antibodies which correlate with immunity. Titers >1:40 or 1:80, as determined by HI tests, are considered protective. The highest rate of infection is reported in pups older than 6 weeks of age. As with other infectious diseases of dogs, puppies from immune bitches are protected for the first weeks of life by maternal antibodies which are acquired via the colostrum. Successful immunization with most vaccines can be accomplished with a high degree of confidence only in seronegative pups, or in pups with very low antibody titers. Maternal antibodies are acquired during the initial 2 - 3 days of life and then decline, with an average half-life of about 9 - 10 days. Passively acquired antibody titers below 40 - 80 are not considered protective against infection, but they commonly interfere with immunization. There is a 'critical period' ('window of vulnerability'), where maternal antibodies are no longer present in sufficient quantity to confer protection. Nevertheless, they may neutralize the vaccine virus, thereby preventing immunization, which is a major problem in achieving successful immunizaton of pups before 12 weeks of age (Fig. 5). In pups from dams which had been infected with virulent parvovirus, maternal antibody interference with vaccination may last as long as 18 - 20 weeks, but >90% of pups from vaccinated populations respond to vaccines at 12 weeks of age. Little is known about cellular immunity in CPV infection, but neutralizing antibodies are known to correlate with protection, and the determination of antibodies therefore allows an assessment of protective immunity.

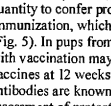

Figure 5. "Critical period" for vaccination against CPV due to the persistence of maternal antibodies. Titers below 1:40 are variably protective, but may interfere with vaccination.To view this image in full size go to the IVIS website at www.ivis.org .

Vaccination of dogs is generally performed using multivalent vaccines which contain canine distemper virus, canine parvovirus, canine adenovirus, leptospira bacterin, and inactivated rabies virus. Monovalent CPV vaccines are also available, some of them containing very high titers (up to 107 TCID50) and are widely advertised for the initial vaccination of puppies. The most common practice in Germany is to vaccinate dogs at 8 weeks of age against CDV, CAV, CPV, and leptospirosis. This schedule is completed by another vaccination at 12 weeks, using CDV, CAV, CPV, leptospira and rabies vaccines. In kennels that have experienced parvovirus problems, vaccination is recommended at 6 weeks of age with a CPV monovalent vaccine.

Both of the schedules, noted above, were tested recently in a study that employed 400 puppies from 60 litters and vaccines from four manufacturers. In that study, about 60% of all puppies seroconverted after a single vaccination, either at 6 weeks of age with a CPV monovalent vaccine, or at 8 weeks of age with a multivalent vaccine. After completion of the immunization series at 12 of age weeks, when all pups had received at two or three inoculations depending on the vaccination schedule used, nearly 10% of the puppies still had not seroconverted. The principal reason for the non-responders was the persistence of interfering levels of maternal antibodies. None of the vaccines tested were capable of breaking through a maternal antibody titer of 1:160 or higher, regardless whether the vaccines were "high titer vaccines" or not. The distribution of maternal antibody titers in pups within a litter was very homogenous, and 97% of the puppies had a maximal titer differences of one 2-fold dilution step, when compared to littermates. In contrast, the decline of maternal antibody titers was not as uniform as expected and serology was not sufficiently accurate to be used as the basis for the estimation of the best time point to achieve successful immunization.

Based on the study above, the following general vaccination schedule is recommended:

1. Vaccination at 6 weeks of age with a CPV monovalent vaccine.
2. Vaccination at 8 weeks of age with a multivalent vaccine containing CPV, CDV, CAV, and leptospira bacterin.
3. Vaccination at 12 weeks of age with a multivalent vaccine containing CPV, CDV, CAV, leptospira bacterin, and rabies virus antigen.
4. Vaccination at 15 or 16 weeks with a multivalent vaccine containing CPV, CDV, CAV, leptospira bacterin and rabies virus antigen.

If it is necessary to develop an individual vaccination schedule, determination of the antibody titer of one or two pups in the litter could be determined at 5 or 6 weeks of age; then, vaccination of the litter may be the calculated on the basis of that titer, using an estimated antibody ½-life of 9.5 days. Vaccination is most likely to be successful when the maternal antibody titer has declined to less than 1:10. Titers below 1:40 are variably protective, but they may interfere with vaccination.

Public Health

Neither CPV nor FPV have been implicated in human disease.

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APPENDIX F

Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix F contains the International Committee on Taxonomy of Viruses' (ICTV). taxonomic structure of the Parvoviridae family, available from the U.S. National Institutes of Health's website at www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm, and was previously provided by Appellant as Exhibit A in their Statement of the Substance of an Interview and After Final Amendment, filed August 4, 2008.

Source:
http://www.ncbi.nlm.nih.gov/ICTVdb

ICTVdB Index of Viruses

Nature of Genome: ssDNA
General Host Type: Vertebrates, Invertebrates
Morphology: not enveloped, isometric

Family 00.050. *Parvoviridae*

Taxonomic Structure of the Family

Family	00.050. *Parvoviridae*
Subfamily	00.050.1. *Parvoririnae*
Genus	00.050.1.01. *Parvovirus*
Genus	00.050.1.02. *Erythrovirus*
Genus	00.050.1.03. *Dependovirus*
Genus	00.050.1.04. *Amdovirus*
Genus	00.050.1.05. *Bocavirus*
Subfamily	00.050.2. *Densovirinae*
Genus	00.050.2.01. *Densovirus*
Genus	00.050.2.02. *Iteravirus*
Genus	00.050.2.03. *Brevidensovirus*
Genus	00.050.2.04. *Pefudensovirus*
	00.050.2.00. Unassigned Species in the Subfamily

Subfamily 00.050.1. *Parvovirinae*

Genus 00.050.1.01. *Parvovirus*

Type Species 00.050.1.01.001. *Minute virus of mice*

List of Species Demarcation Criteria in the Genus

Members of each species are antigenically distinct, as assessed by neutralization using polyclonal antisera, and natural infection is usually confined to a single host species. Generally, species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.01.006.	*Chicken parvovirus*		
00.050.1.01.006.00.001.	Chicken parvovirus		(ChPV)
00.050.1.01.007.	*Feline panleukopenia virus*		
00.050.1.01.007.00.005.	Canine parvovirus	[M19296]	(CPV)
00.050.1.01.007.00.007.	Feline panleukopenia virus	[M75728]	(FPV)
00.050.1.01.007.00.015.	Mink enteritis virus	[D00765]	(MEV)
00.050.1.01.007.00.018.	Raccoon parvovirus	[M24005]	(RPV)
00.050.1.01.010.	*HB parvovirus*		
00.050.1.01.010.00.001.	HB parvovirus		(HBPV)
00.050.1.01.011.	*H-1 parvovirus*		
00.050.1.01.011.00.001.	H-1 parvovirus	[X01457]	(H-1PV)
00.050.1.01.012.	*Kilham rat virus*		
00.050.1.01.012.00.002.	H-3 virus		
00.050.1.01.012.00.001.	Kilham rat virus	[AF321230]	(KRV)
00.050.1.01.012.00.001.	(Rat virus)	[AF321230]	(KRV)
00.050.1.01.013.	*Lapine parvovirus*		
00.050.1.01.013.00.001.	Lapine parvovirus		(LPV)
00.050.1.01.014.	*LuIII virus*		
00.050.1.01.014.00.001.	LuIII virus	[M81888]	(LuIIIV)
00.050.1.01.016.	*Minute virus of mice*		
00.050.1.01.016.00.003.	Minute virus of mice (Cutter)	[U34256]	(MVMc)
00.050.1.01.016.00.003.	(Mice minute virus)	[U34256]	
00.050.1.01.016.00.002.	Minute virus of mice	[M12032]	(MVMi)

	(immunosuppressive)		
00.050.1.01.016.00.001.	Minute virus of mice (prototype)	[J02275]	(MVMp)
00.050.1.01.021.	*Mouse parvovirus 1*		
00.050.1.01.021.00.001.	Mouse parvovirus 1	[U12469]	(MPV-1)
00.050.1.01.017.	*Porcine parvovirus*		
00.050.1.01.017.00.001.	Porcine parvovirus Kresse	[U44978]	(PPV-Kr)
00.050.1.01.017.00.002.	Porcine parvovirus NADL-2	[L23427]	(PPV-NADL2)
00.050.1.01.019.	*RT parvovirus*		
00.050.1.01.019.00.001.	RT parvovirus		(RTPV)
00.050.1.01.020.	*Tumor virus X*		
00.050.1.01.020.00.001.	Tumor virus X		(TVX)

Tentative Species in the Genus

00.050.1.81.026.	Hamster parvovirus		
00.050.1.81.026.00.001.	Hamster parvovirus	[U34255]	(HaPV)
00.050.1.81.028.	Rat minute virus 1		
00.050.1.81.028.00.001.	Rat minute virus 1	[AF332882]	(RMV-1)
00.050.1.81.027.	Rat parvovirus 1		
00.050.1.81.027.00.001.	Rat parvovirus 1	[AF036710]	(RPV-1)
00.050.1.81.023.	Rheumatoid arthritis virus		

Genus 00.050.1.02. *Erythrovirus*

Type Species 00.050.1.02.001. *Human parvovirus B19*

List of Species Demarcation Criteria in the Genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.02.001.	*Human parvovirus B19*		
00.050.1.02.001.00.001.	Human parvovirus B19 - A6	[AY064475]	(B19V-A6)
00.050.1.02.001.00.001.	Human parvovirus B19 - A6	[AY064476]	
00.050.1.02.001.00.002.	Human parvovirus B19 - Au	[M13178]	(B19V-Au)
00.050.1.02.001.00.003.	Human parvovirus B19 - LaLi	[AY044266]	(B19V-LaLi)
00.050.1.02.001.00.004.	Human parvovirus B19 - V9	[AJ223617]	(B19V-V9)
00.050.1.02.001.00.004.	Human parvovirus B19 - V9	[AJ242810]	
00.050.1.02.001.00.005.	Human parvovirus B19 - Wi	[M24682]	(B19V-Wi)
00.050.1.02.004.	*Pig-tailed macaque parvovirus*		
00.050.1.02.004.00.001.	Pig-tailed macaque parvovirus	[AF221123]	(PMPV)
00.050.1.02.005.	*Rhesus macaque parvovirus*		
00.050.1.02.005.00.001.	Rhesus macaque parvovirus	[AF221122]	(RmPV)
00.050.1.02.002.	*Simian parvovirus*		
00.050.1.02.002.00.001.	Simian parvovirus (cynomolgus)	[U26342]	(SPV)

Tentative Species in the Genus

00.050.1.82.006.	Bovine parvovirus type 3		
00.050.1.82.006.00.001.	Bovine parvovirus type 3	[AF406967]	(BPV-3)
00.050.1.82.003.	Chipmunk parvovirus		
00.050.1.82.003.00.001.	Chipmunk parvovirus	[U86868]	(ChpPV)

Genus 00.050.1.03. *Dependovirus*

Type Species 00.050.1.03.001. *Adeno-associated virus - 2*

List of Species Demarcation Criteria in the Genus

Members of each species are antigenically distinct, as assessed by neutralization using polyclonal antisera, and natural infection is usually confined to a single host species. Generally, species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.03.002.	*Adeno-associated virus - 1*		
00.050.1.03.002.00.001.	Adeno-associated virus - 1	[AF063497]	(AAV-1)
00.050.1.03.002.00.006.	Adeno-associated virus - 6	[AF208704]	(AAV-6)
00.050.1.03.003.	*Adeno-associated virus - 2*		
00.050.1.03.003.00.001.	Adeno-associated virus - 2	[J01901]	(AAV-2)
00.050.1.03.004.	*Adeno-associated virus - 3*		
00.050.1.03.004.00.001.	Adeno-associated virus - 3	[AF028705]	(AAV-3)
00.050.1.03.005.	*Adeno-associated virus - 4*		
00.050.1.03.005.00.001.	Adeno-associated virus - 4	[U89790]	(AAV-4)
00.050.1.03.006.	*Adeno-associated virus - 5*		
00.050.1.03.006.00.001.	Adeno-associated virus - 5	[AF085716]	(AAV-5)
00.050.1.03.007.	*Avian adeno-associated virus*		
00.050.1.03.007.00.001.	Avian adeno-associated virus	[AY186198]	(AAAV)
00.050.1.03.008.	*Bovine adeno-associated virus*		
00.050.1.03.008.00.001.	Bovine adeno-associated virus		(BAAV)
00.050.1.03.009.	*Canine adeno-associated virus*		
00.050.1.03.009.00.001.	Canine adeno-associated virus		(CAAV)
00.050.1.03.014.	*Duck parvovirus*		
00.050.1.03.014.00.001.	Barbarie duck parvovirus	[U22967]	(BDPV)
00.050.1.03.014.00.022.	Muscovy duck parvovirus	[X75093]	(MDPV)
00.050.1.03.010.	*Equine adeno-associated virus*		
00.050.1.03.010.00.001.	Equine adeno-associated virus		(EAAV)
00.050.1.03.013.	*Goose parvovirus*		
00.050.1.03.013.00.001.	Goose parvovirus	[U25749]	(GPV)
00.050.1.03.011.	*Ovine adeno-associated virus*		
00.050.1.03.011.00.001.	Ovine adeno-associated virus		(OAAV)

Tentative Species in the Genus

00.050.1.83.014.	Adeno-associated virus 7		
00.050.1.83.014.00.001.	Adeno-associated virus - 7	[AF513851]	(AAV-7)
00.050.1.83.014.	(Adeno-associated virus-7)		
00.050.1.83.015.	Adeno-associated virus 8		
00.050.1.83.015.00.001.	Adeno-associated virus - 8	[AF513852]	(AAV-8)
00.050.1.83.015.	(Adeno-associated virus-8)		
00.050.1.83.016.	Bovine parvovirus 2		
00.050.1.83.016.00.001.	Bovine parvovirus - 2	[AF406966]	(BPV-2)
00.050.1.83.016.	(Bovine parvovirus-2)		

Genus 00.050.1.04. *Amdovirus*

Type Species 00.050.1.04.001. *Aleutian mink disease virus*

List of Species Demarcation Criteria in the Genus

Not applicable.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.04.001.	*Aleutian mink disease virus*		
00.050.1.04.001.00.001.	Aleutian mink disease virus	[M20036]	(AMDV)
00.050.1.04.001.	(Aleutian disease virus)		

Tentative Species in the Genus

None reported.

Genus 00.050.1.05. *Bocavirus*

Type Species 00.050.1.05.001. *Bovine parvovirus*

List of Species Demarcation Criteria in the Genus

Members of each species are probably antigenically distinct and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.1.05.001.	*Bovine parvovirus*		
00.050.1.05.001.00.001.	Bovine parvovirus	[M14363]	(BPV)
00.050.1.05.002.	*Canine minute virus*		
00.050.1.05.002.00.001.	Canine minute virus	[AF495467]	(CnMV)

Tentative Species in the Genus

None reported.

Subfamily 00.050.2. *Densovirinae*

Genus 00.050.2.01. *Densovirus*

Type Species 00.050.2.01.001. *Junonia coenia densovirus*

List of Species Demarcation Criteria in the Genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.2.01.002.	*Galleria mellonella densovirus*		
00.050.2.01.002.00.001.	Galleria mellonella densovirus	[L32896]	(GmDNV)
00.050.2.01.001.	*Junonia coenia densovirus*		
00.050.2.01.001.00.001.	Junonia coenia densovirus	[S17265]	(JcDNV)

Tentative Species in the Genus

00.050.2.81.008.	Diatraea saccharalis densovirus		
00.050.2.81.008.00.001.	Diatraea saccharalis densovirus	[AF036333]	(DsDNV)
00.050.2.81.006.	Mythimna loreyi densovirus		
00.050.2.81.006.00.001.	Mythimna loreyi densovirus	[AY461507]	(MlDNV)
00.050.2.81.007.	Pseudoplusia includens densovirus		
00.050.2.81.007.00.001.	Pseudoplusia includens densovirus		(PiDNV)
00.050.2.81.009.	Toxorhynchites splendens densovirus		
00.050.2.81.009.00.001.	Toxorhynchites splendens densovirus	[AF395903]	(TsDNV)

Genus 00.050.2.02. *Iteravirus*

Type Species 00.050.2.02.001. *Bombyx mori densovirus*

List of Species Demarcation Criteria in the Genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.2.02.001.	*Bombyx mori densovirus*		
00.050.2.02.001.00.001.	Bombyx mori densovirus	[AY033435]	(BmDNV)

Tentative Species in the Genus

00.050.2.82.004.	Casphalia extranea densovirus		
00.050.2.82.004.00.001.	Casphalia extranea densovirus	[AF375296]	(CeDNV)
00.050.2.82.005.	Sibine fusca densovirus		
00.050.2.82.005.00.001.	Sibine fusca densovirus		(SfDNV)

Genus 00.050.2.03. *Brevidensovirus*

Type Species 00.050.2.03.001. *Aedes aegypti densovirus*

List of Species Demarcation Criteria in the Genus

Members of each species are probably antigenically distinct, and natural infection is confined to a single host species. Species are <95% related by non-structural gene DNA sequence.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.2.03.001.	*Aedes aegypti densovirus*		
00.050.2.03.001.00.001.	Aedes aegypti densovirus	[AY160976]	(AaeDNV)
00.050.2.03.002.	*Aedes albopictus densovirus*		
00.050.2.03.002.00.001.	Aedes albopictus densovirus	[AY095351]	(AalDNV)

Tentative Species in the Genus

00.050.2.83.015.	Aedes pseudoscutellaris densovirus		
00.050.2.83.015.00.001.	Aedes pseudoscutellaris densovirus		(ApDNV)
00.050.2.83.003.	Agraulis vanillae densovirus		
00.050.2.83.008.	Lymantria dubia densovirus		
00.050.2.83.019.	Penaeus stylirostris densovirus		
00.050.2.83.019.00.001.	Penaeus stylirostris densovirus	[AF273215]	(PstDNV)
00.050.2.83.011.	Pseudaletia includens densovirus		
00.050.2.83.012.	Sibine fusca densovirus		
00.050.2.83.013.	Simulium vittatum densovirus		
00.050.2.83.013.00.001.	Simulium vittatum densovirus		(SvDNV)

Genus 00.050.2.04. *Pefudensovirus*

Type Species 00.050.2.04.001. *Periplaneta fuliginosa densovirus*

List of Species Demarcation Criteria in the Genus

Not applicable.

List of Species in the Genus

The ICTVdB virus code and the viruses. Official virus *species names* are in italics. Tentative virus species names, alternative names ( ), isolates, strains, serotypes, subspecies, or rejected names are not italicized.

Virus codes, virus names, genome sequence accession numbers [ ] and assigned abbreviations ( ), are:

Species, their serotypes, strains and isolates

00.050.2.04.001.	*Periplaneta fuliginosa densovirus*		
00.050.2.04.001.00.001.	Periplaneta fuliginosa densovirus	[AF192260]	(PfDNV)

Tentative Species in the Genus

None reported.

Unassigned Species in the Subfamily

00.050.2.00.014.	Acheta domestica densovirus		
00.050.2.00.014.00.001.	Acheta domesticus densovirus	[AX344110]	(AdDNV)
00.050.2.00.015.	Blattella germanica densovirus		
00.050.2.00.015.00.001.	Blattella germanica densovirus	[AY189948]	(BgDNV)
00.050.2.00.008.	Culex pipiens densovirus		
00.050.2.00.008.00.001.	Culex pipiens densovirus		(CpDNV)
00.050.2.00.016.	Euxoa auxiliaris densovirus		
00.050.2.00.016.00.001.	Euxoa auxiliaris densovirus		(EaDNV)
00.050.2.00.001.	Hepatopancreatic parvo-like virus of shrimps		
00.050.2.00.017.	Leucorrhinia dubia densovirus		
00.050.2.00.017.00.001.	Leucorrhinia dubia densovirus		(LduDNV)
00.050.2.00.018.	Lymantria dispar densovirus		
00.050.2.00.018.00.001.	Lymantria dispar densovirus		(LdiDNV)
00.050.2.00.003.	Myzus persicae densovirus		
00.050.2.00.003.00.001.	Myzus persicae densovirus	[AY148187]	(MpDNV)
00.050.2.00.002.	Parvo-like virus of crabs		
00.050.2.00.019.	Pieris rapae densovirus		
00.050.2.00.019.00.001.	Pieris rapae densovirus		(PrDNV)
00.050.2.00.004.	Planococcus citri densovirus		
00.050.2.00.004.00.001.	Planococcus citri densovirus	[AY032882]	(PcDNV)

List of Unassigned Viruses in the Family

None reported.

Similarity with other Taxa

None reported.

Derivation of Names

Amdo: sigla from *A*leutian *M*ink *D*isease. *Boca*: sigla from *Bo*vine and *Ca*nine *Brevi*: from Latin *brevis*, "short". *Denso*: from Latin *densus*, "thick, compact". *Dependo*: from Latin *dependeo*, "to hang down". *Erythro*: from Greek *erythros*, "red". *Itera*: from Latin *iterum*, "again", "repeat" *Parvo*: from Latin *parvus*, "small". *Pefu*: sigla from *Pe*riplaneta *fu*liginosa densovirus, type species of the *Pefudensovirus* genus.

References

Collated from VIIIth ICTV Report

Contributed by

Tattersall, P., Bergoin, M., Bloom, M.E., Brown, K.E., Linden, R.M., Muzyczka, N., Parrish, C.R. and Tijssen, P.

Addtional References

Reference List from the 8th ICTV Report
ICTVdB taxon description
ICTVdB Picture Gallery

References to sequence databases at GenBank and PubMed Central:
PubMed Central References; nucleotide sequences; complete genomes

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Source: http://www.ncbi.nlm.nih.gov/ICTVdb/Ictv/index.htm

APPENDIX G

Evidence Appendix (37 C.F.R. §41.37(c)(1)(ix))

Appendix G contains a definition for the Latin prefix "parvo," available from www.wordinfo.info. A printout of this definition was previously provided as Exhibit B of Appellant's August 4, 2008 filing.

Source:
http://www.wordinfo.info/words/index/info/view_unit/1582/2/?page=&letter=

parvo-, parvi- +

(Latin: [*parvus*] small, little; minute, minuscule)

Little things contribute to perfection, and perfection is not a little thing.

—John Rayoa

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parvoviral
Caused by or relating to a parvovirus.

parvovirus
1. Any of a group of very small animal viruses consisting of some single-stranded DNA in an icosahedral capsid without an envelope and occurring in a wide variety of vertebrates, often as a pathogen.
2. In veterinary medicine, an often fatal disease of dogs caused by a parvovirus, characterized by diarrhea and vomiting.
3. Parvoviruses; a genus of viruses of the subfamily *Parvovirinae* (family *Parvoviridae*) that infect mammals and birds. Viruses multiply in the nucleus and require S-phase

cellular functions for replication.

Bovine parvovirus, a virus of the genus *Parvovirus* infecting cattle that causes diarrhea in calves; infection during the first or second trimesters of gestation may result in abortion. Infection is widespread and antibody to the virus can be found in a high proportion of adult cattle.

Canine parvovirus, a virus of the genus *Parvovirus* that causes myocarditis in dogs and a type of enteritis called canine parvovirus disease; it is sometimes considered to be a species-specific variant of feline parvovirus.

Feline parvovirus, a virus of the genus *Parvovirus* that primarily affects cats. Canine parvovirus, feline panleukopenia virus, and mink enteritis virus are sometimes considered to be host-specific variant strains.

Goose parvovirus, a virus of the genus *Parvovirus* that causes a highly fatal disease of young geese affecting the liver, thyroid, and pancreas.

Porcine parvovirus, A worldwide virus of pigs that has been associated with infertility and abortion.

Transmission is transplacental or by mechanical vector. Human parvoviruses cause transient aplastic crisis, acute arthritis, erythema infectiosum, hydrops fetalis, spontaneous abortion, and fetal death.

Animal pathogens include such animals as: bovine, canine, feline, and goose parvoviruses, feline panleukopenia virus, mink enteritis virus, Aleutian mink disease virus, and various murine parvoviruses.

parvule

In pharmacology, a very small pill, pellet, or granule.

parvum (small) **opus** (work)

Small work as opposed to magnum (big, great) opus (work).

If there are any numbers below, use them to see other pages in this unit.

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Showing page 2 out of 2 pages of 19 words.

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The Main-Word Info page

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APPENDIX H
Related Proceedings Appendix (37 C.F.R. §41.37(c)(1)(x))

none